US009226953B2

(12) **United States Patent**
Zwaal

(10) **Patent No.:** **US 9,226,953 B2**
(45) **Date of Patent:** **Jan. 5, 2016**

(54) **VARIANTS OF PLASMINOGEN AND PLASMIN**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 498 days.

(21) Appl. No.: **13/383,086**

(22) PCT Filed: **Jul. 9, 2010**

(86) PCT No.: **PCT/EP2010/059902**

§ 371 (c)(1), (2), (4) Date: **Jan. 11, 2012**

(87) PCT Pub. No.: **WO2011/004011**

PCT Pub. Date: **Jan. 13, 2011**

(65) **Prior Publication Data**

US 2012/0114630 A1 May 10, 2012

Related U.S. Application Data

(60) Provisional application No. 61/224,514, filed on Jul. 10, 2009.

(30) **Foreign Application Priority Data**

Jul. 10, 2009 (EP) 09165237

(51) **Int. Cl.**
A61K 38/00 (2006.01)
A61K 38/48 (2006.01)
C12N 9/68 (2006.01)

(52) **U.S. Cl.**
CPC ***A61K 38/484*** (2013.01); ***C12N 9/6435*** (2013.01); ***C12Y 304/21007*** (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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(57) **ABSTRACT**

The invention relates to variants of plasminogen and plasmin comprising one or more point mutations in the catalytic domain which reduce or prevent autocatylic destruction of the protease activity of plasmin. Compositions, uses and methods of using said variants of plasminogen and plasmin are also disclosed.

46 Claims, 24 Drawing Sheets

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```
1          11         21         31         41         51
|          |          |          |          |          |
EPLDDYVNTQ GASLFSVTKK QLGAGSIEEC AAKCEEDEEF TCRAFQYHSK EQQCVIMAEN

61         71         81         91         101        111
|          |          |          |          |          |
RKSSIIIRMR DVVLFEKKVY LSECKTGNGK NYRGTMSKTK NGITCQKWSS TSPHRPRFSP

121        131        141        151        161        171
|          |          |          |          |          |
ATHPSEGLEE NYCRNPDNDP QGPWCYTTDP EKRYDYCDIL ECEEECMHCS GENYDGKISK

181        191        201        211        221        231
|          |          |          |          |          |
TMSGLECQAW DSQSPHAHGY IPSKFPNKNL KKNYCRNPDR ELRPWCFTTD PNKRWELCDI

241        251        261        271        281        291
|          |          |          |          |          |
PRCTTPPPSS GHTYQCLKGT GENYRGNVAV TVSGHTCQHW SAQTPHTHNR TPENFPCKNL

301        311        321        331        341        351
|          |          |          |          |          |
DENYCRNPDG KRAPWCHTTN SQVRWEYCKI PSCDSSPVST EQLAPTAPPE LTPVVQDCYH

361        371        381        391        401        411
|          |          |          |          |          |
GDGQSYRGTS STTTTGKKCQ SWSSMTPHRH QKTPENYPNA GLTMNYCRNP DADKGPWCFT

421        431        441        451        461        471
|          |          |          |          |          |
TDPSVRWEYC NLKKCSGTEA SVVAPPPVVL LPDVETPSEE DCMFGNGKGY RGKRATTVTG

481        491        501        511        521        531
|          |          |          |          |          |
TPCQDWAAQE PHRHSIFTPE TNPRAGLEKN YCRNPDGDVG GPWCYTTNPR KLYDYCDVPQ

541        551        561        571        581        591
|          |          |1        9|        19|        29|        39
|          |          ||        ||         ||         ||         |
CAAPSFDCGK PQVEPKKCPG RVVGGCVAHP HSWPWQVSLR TRFGMHFCGG TLISPEWVLT

601        611        621        631        641        651
|        49|        59|        69|        79|        89|        99
|         ||         ||         ||         ||         ||         |
AAHCLEKSPR PSSYKVILGA HQEVNLEPHV QEIEVSRLFL EPTRKDIALL KLSSPAVITD

661        671        681        691        701        711
|       109|       119|       129|       139|       149|       159
|         ||         ||         ||         ||         ||         |
KVIPACLPSP NYVVADRTEC FITGWGETQG TFGAGLLKEA QLPVIENKVC NRYEFLNGRV

721        731        741        751        761        771
|       169|       179|       189|       199|       209|       219
|         ||         ||         ||         ||         ||         |
QSTELCAGHL AGGTDSCQGD SGGPLVCFEK DKYILQGVTS WGLGCARPNK PGVYVRVSRF

781        791
|       229|
|         ||
VTWIEGVMRN N (SEQ ID NO:1)
```

FIGURE 1

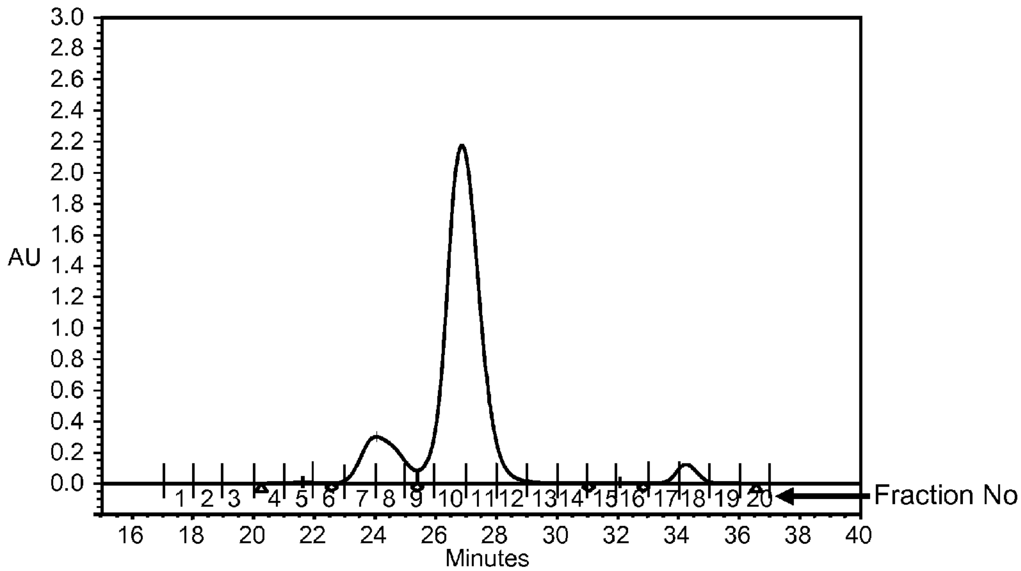

3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
AU
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Fraction No
16 18 20 22 24 26 28 30 32 34 36 38 40
Minutes

FIGURE 2

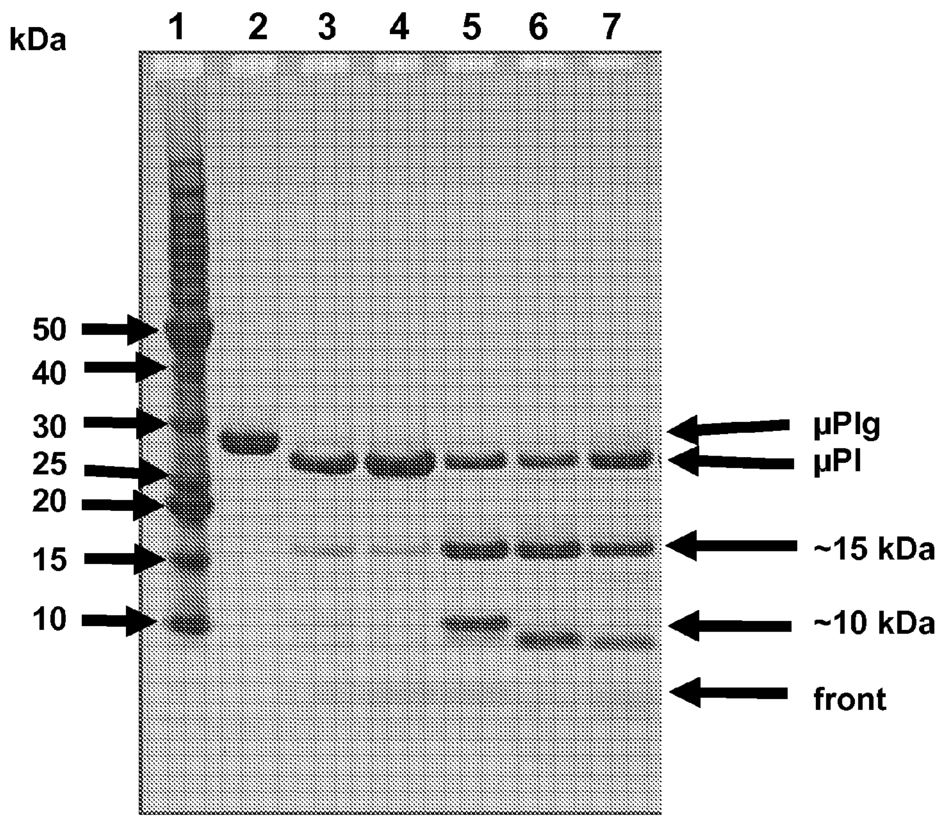


FIGURE 3

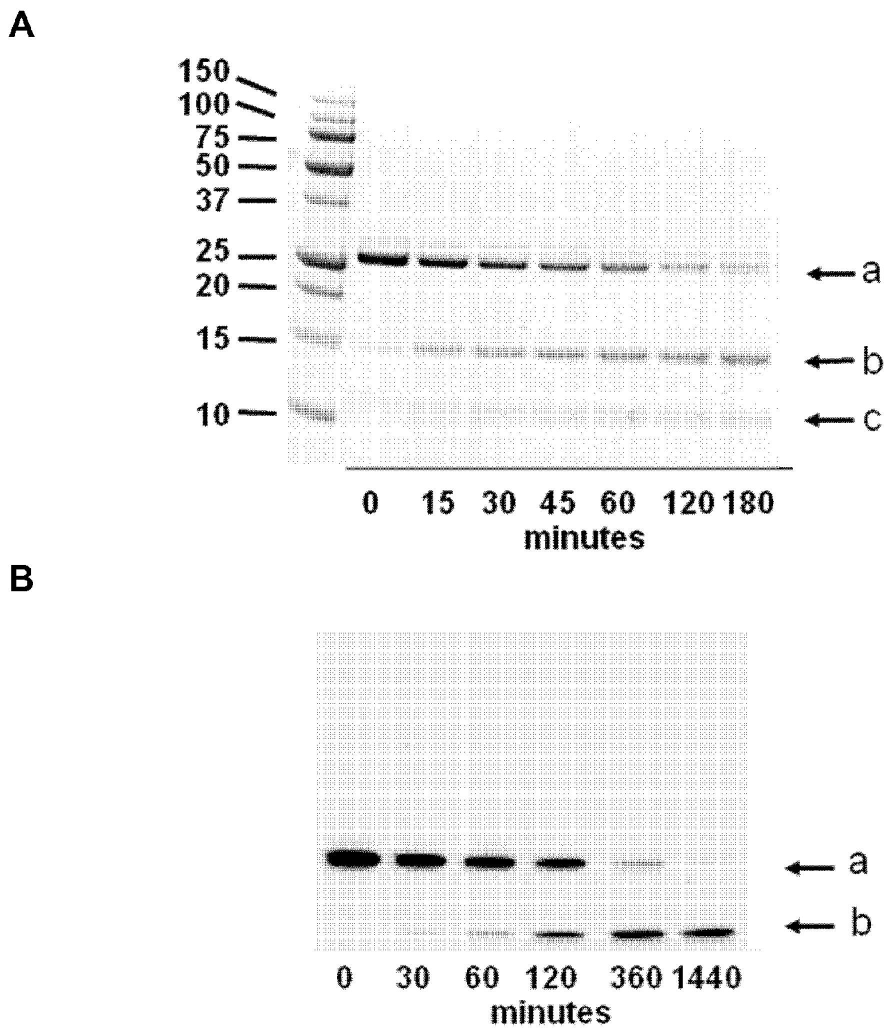

A
150
100
75
50
37
25
20
15
10
a
b
c
0 15 30 45 60 120 180
minutes
B
a
b
0 30 60 120 360 1440
minutes

FIGURE 4

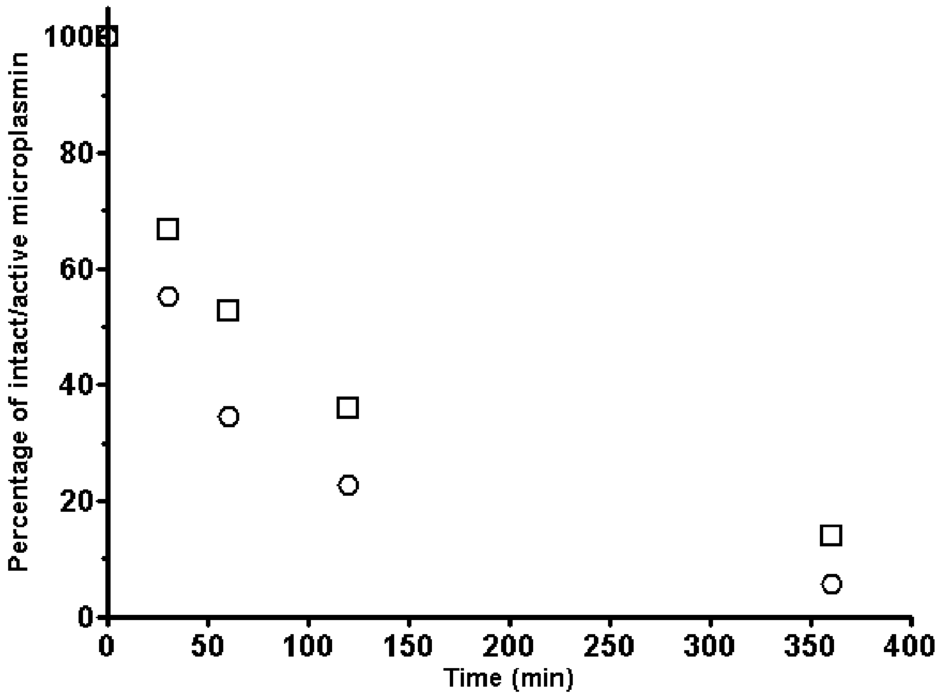

100
80
60
40
20
0
Percentage of intact/active microplasmin
0
50
100
150
200
250
300
350
400
Time (min)

FIGURE 5

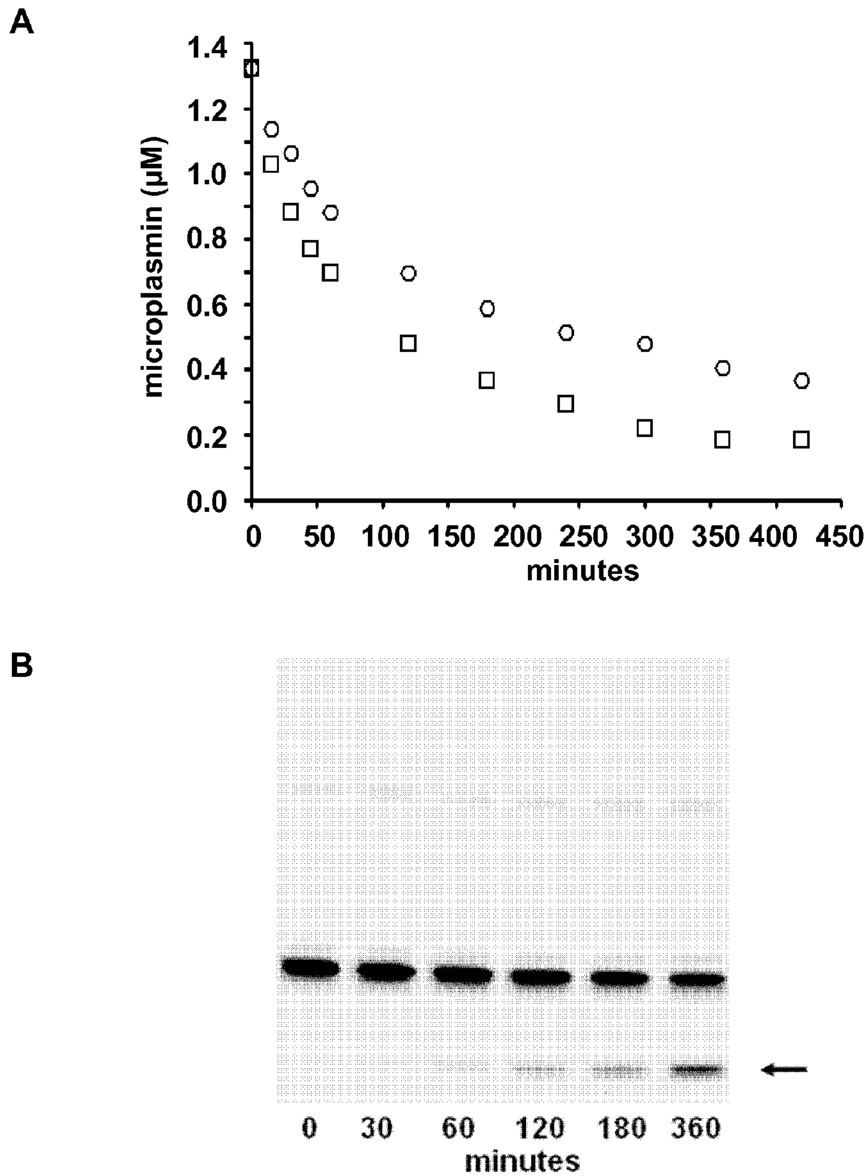

A
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
microplasmin (µM)
0
50
100
150
200
250
300
350
400
450
minutes
B
0
30
60
120
180
360
minutes

FIGURE 6

A

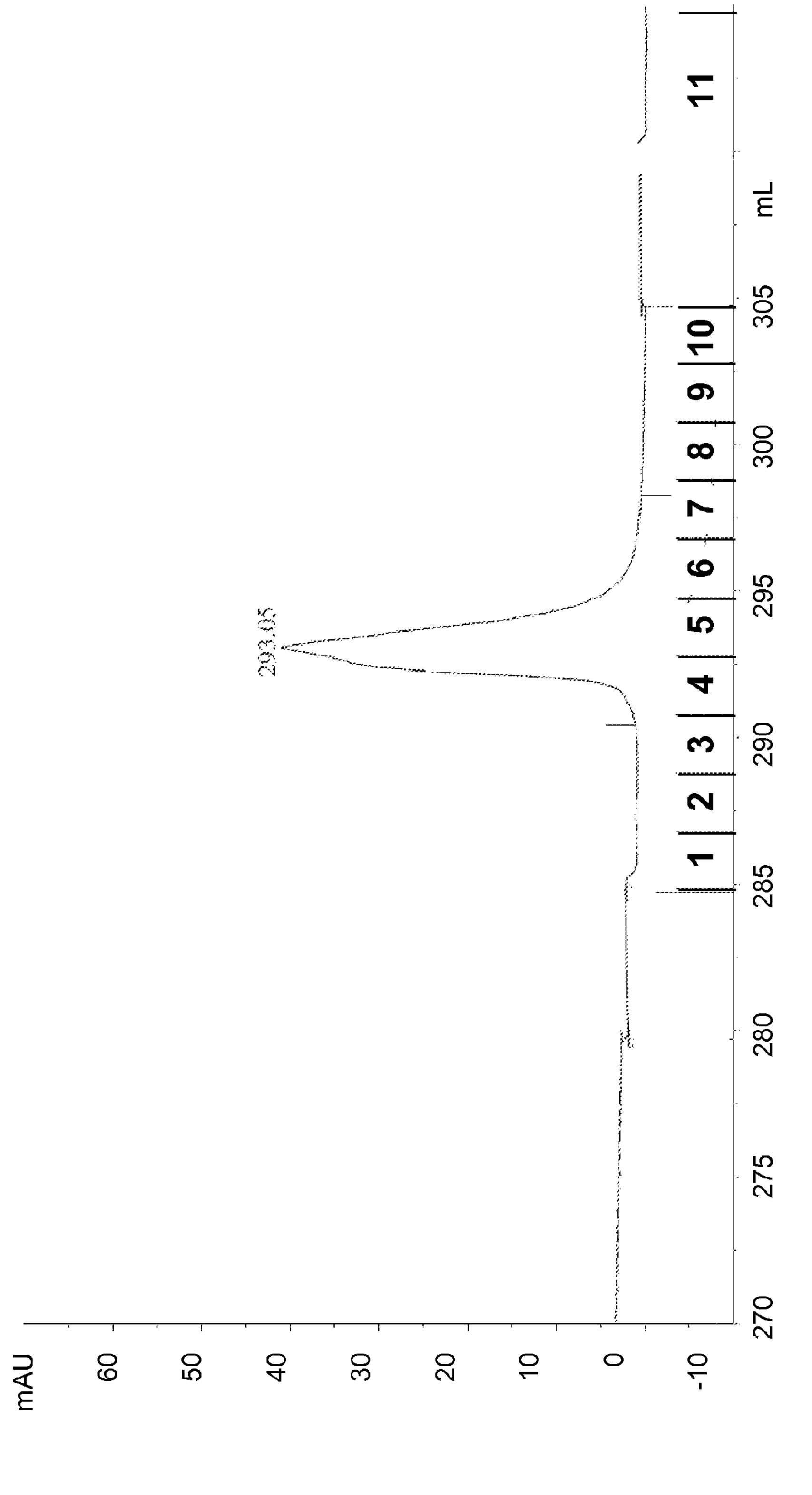

mAU
60
50
40
30
20
10
0
-10
270
275
280
285
290
295
300
305
mL
293.05
1
2
3
4
5
6
7
8
9
10
11

FIGURE 7/1

B

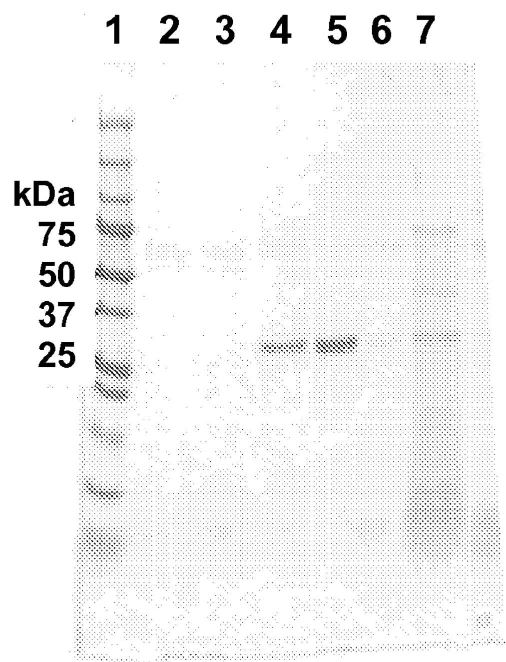
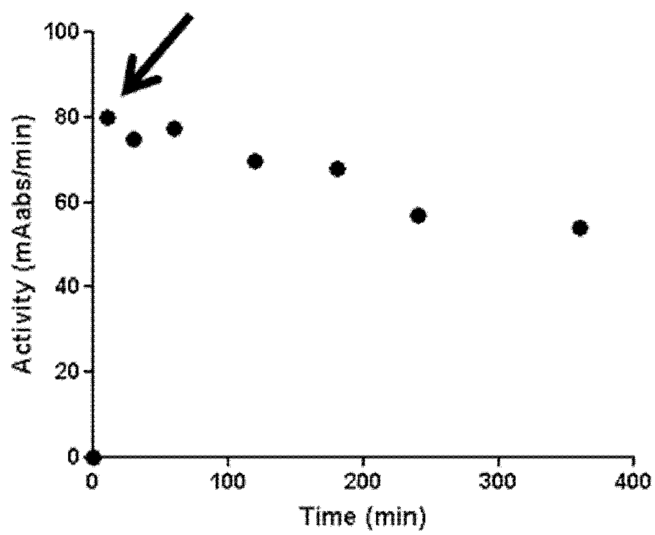


FIGURE 7/2

A


Activity (mAabs/min)
0
20
40
60
80
100
0
100
200
300
400
Time (min)

B

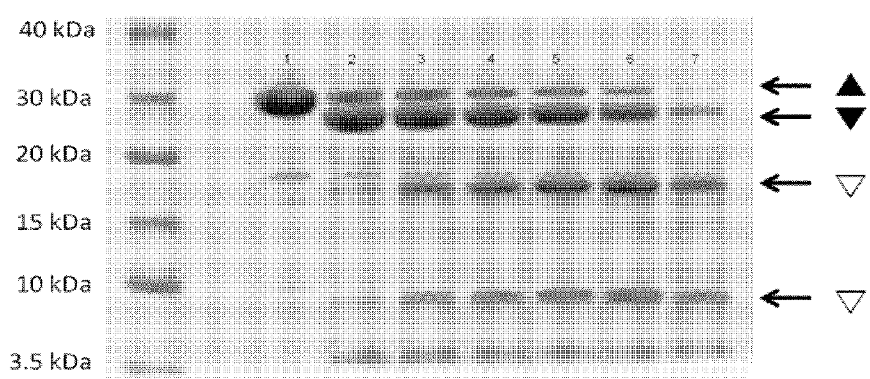

40 kDa
30 kDa
20 kDa
15 kDa
10 kDa
3.5 kDa

FIGURE 8/1

C

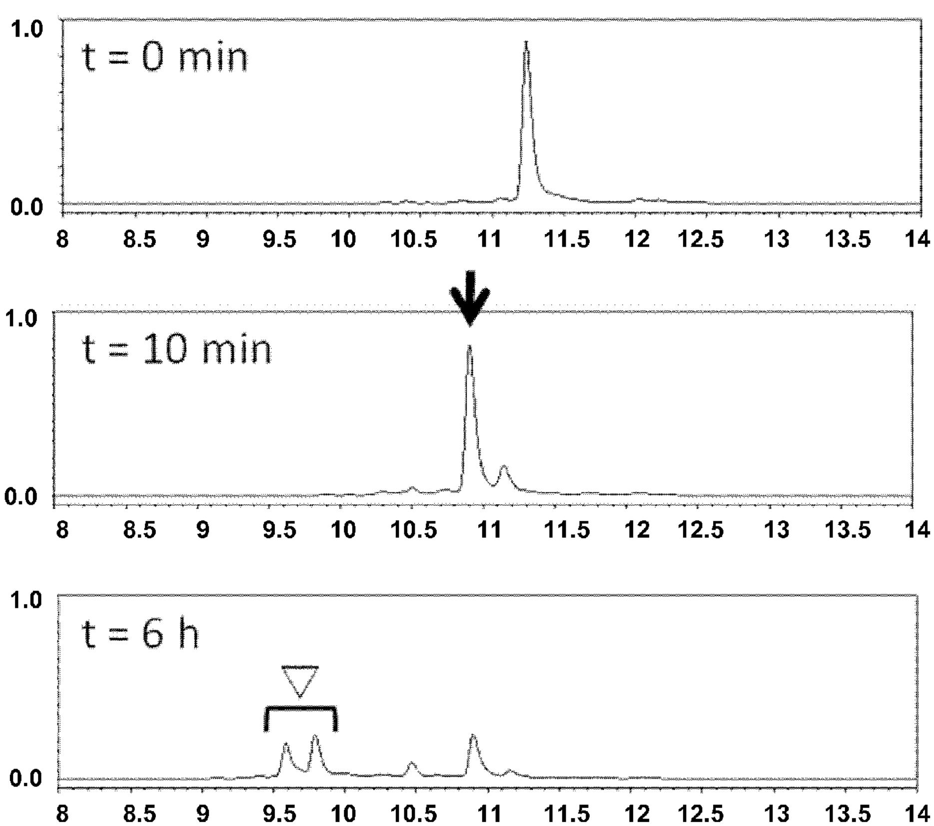

1.0
t = 0 min
0.0
8
8.5
9
9.5
10
10.5
11
11.5
12
12.5
13
13.5
14
1.0
t = 10 min
0.0
8
8.5
9
9.5
10
10.5
11
11.5
12
12.5
13
13.5
14
1.0
t = 6 h
0.0
8
8.5
9
9.5
10
10.5
11
11.5
12
12.5
13
13.5
14

FIGURE 8/2

D

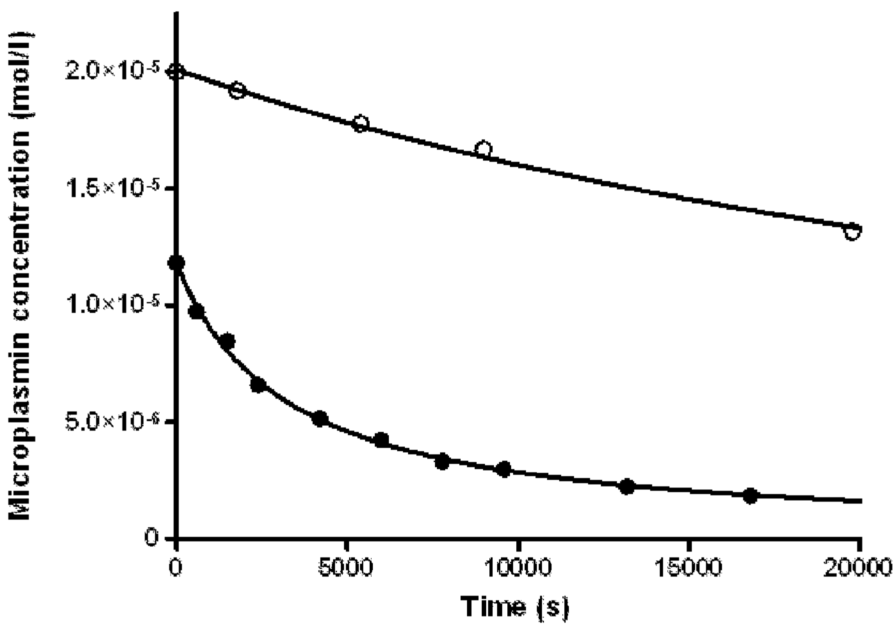

Microplasmin concentration (mol/l)
2.0×10-5
1.5×10-5
1.0×10-5
5.0×10-6
0
0
5000
10000
15000
20000
Time (s)

FIGURE 8/3

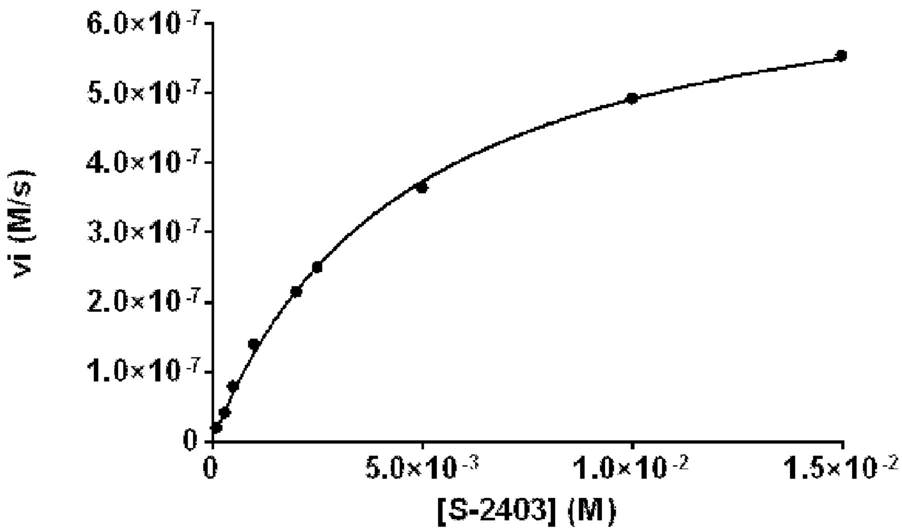

6.0×10-7
5.0×10-7
4.0×10-7
3.0×10-7
2.0×10-7
1.0×10-7
0
vi (M/s)
0
5.0×10-3
1.0×10-2
1.5×10-2
[S-2403] (M)

FIGURE 9

COBALT (Constraint-based Multiple Alignment Tool) alignment of plasminogen amino acid sequences

Line # in sequence alignment

1: Homo sapiens / Genbank NP_000292.1/ human (SEQ ID NO:50)
2: Canis familiaris /Genbank XP_533468/ dog (SEQ ID NO:26)
3: Pan troglodytes /Genbank XP_001152889/ chimpanzee/ isoform 3 (SEQ ID NO:27)
4: Pan troglodytes /Genbank XP_001152830/ chimpanzee/ isoform 2 (SEQ ID NO:28)
5: Pan troglodytes /Genbank XP_518844/ chimpanzee/ isoform 4 (SEQ ID NO:29)
6: Macaca mulatta /Genbank NP_001036540/ Rhesus monkey (SEQ ID NO:30)
7: Pongo abelii /Genbank NP_001126035/ Sumatran orangutan (SEQ ID NO:31)
8: Sus scrofa /Genbank NP_001038055/ pig (SEQ ID NO:32)
9: Bos Taurus /Genbank DAA25966/ cattle (SEQ ID NO:33)
10: Equus caballus /Genbank XP_001500552/ horse (SEQ ID NO:34)
11: Mus musculus /Genbank EDL02061/ house mouse (SEQ ID NO:35)
12: Rattus norvegicus /Genbank NP_445943/ Norway rat (SEQ ID NO:36)
13: Erinaceus europaeus /Genbank AAC48717/ western European hedgehog (SEQ ID NO:37)
14: Oryctolagus cuniculus /Genbank XP_002715012/ rabbit (SEQ ID NO:38)
15: Pan troglodytes/ Genbank XP_001152435/ chimpanzee/ isoform 1 (SEQ ID NO:39)
16: Ailuropoda melanoleuca/ Genbank EFB19688/panda (SEQ ID NO:40)
17: Papio hamadryas/ Genbank AAB97887/baboon (SEQ ID NO:41)
18: Ovis aries/ Genbank P81286/sheep (SEQ ID NO:42)

FIGURE 10/1

```
                                  1         11        21        31        41
                                 ▼|         |         |         |         |
1  1   -------MEHKEVVLLLLLFLKSGQG EPLDDYVNTQGASLFSVTKKQLGAGSIEECAAKCEEDEEFTCR-----------
2  1   -------MEHKEVVLLLLLFLKSGHG SLLDDYVNTQGASVFSLTKKQLSVGSIEECAAKCEEETGFICR-----------
3  1   -------MEHKEVVLLLLLFLKSGQG EPLDDYVNTQGASLFSVTKKQLGAGSIEECAAKCEEDKEFTCR-----------
4  1   -------MEHKEVVLLLLLFLKSGQG EPLDDYVNTQGASLFSVTKKQLGAGSIEECAAKCEEDKEFTCR-----------
5  1   -----------------MLMDYEGQG EPLDDYVNTQGASLFSVTKKQLGAGSIEECAAKCEEDKEFTCR-----------
6  1   -------MEHKEVVLLLLLFLKSGQG EPLDDYVNTKGASLFSITKKQLGAGSIEECAAKCEEEEEFTCR-----------
7  1   -------MEHKEVVLLLLLFLKSGQG EPLDDYVNTQGASLFSVTKKQLRAGSIEECAAKCEEEKEFTCR-----------
8  1   -------MDHKEVVLLLLLFLKSGLG DSLDDYVNTQGAFLFSLSRKQVAARSVEECAAKCEAETNFICR-----------
9  1   MLPASPKMEHKAVVFLILLFLKSGLG DLLDDYVNTQGASLLSLSRKNLAGRSVEDCAAKCEEETDFVCR-----------
10 1   -------MEHQEVVFLLLLFLKSGHG DILDDYVTTQGASLFTFTRKPLSASSIEECEAKCTEETAFICR-----------
11 1   -------MDHKEVILLFLLLLKPGQG DSLDGYISTQGASLFSLTKKQLAAGGVADCLAKCEGETDFVCR-----------
12 1   -------MDHKEIILLFLLFLKPGQG DSLDGYVSTQGASLHSLTKKQLAAGSIADCLAKCEGETDFICR-----------
13 1   -------MQRKELVLLFLLFLQPGHG IPLDDYVTTQGASLSSSTKKQLSVGSTEECAVKCEKETSFICR-----------
14 1   -------MEQRAVVLLLLLLLKPGQA EPLDDYVNTQGASLFSFTKKQLGAASIAECAARCEAETEFTCR-----------
15 1   -------MEHKEVVLLLLLFLKSGQG EPLDDYVNTQGASLFSVTKKQLGAGSIEECAAKCEEDKEFTCRYFHCRCTYPEI
16 1   -------------------------- ---------------------------------------FVRR-----------
```

FIGURE 10/2

```
                  51        61        71        81        91        101       111
                  |         |         |         |         |         |         |
1   63   ------AFQYHSKEQQCVIMAENRKSSIIIRMRDVVLFEKKVYLSECKTGNGKNYRGTMSKTKNGITCQKWSSTSPHRPR
2   63   ------SFQYHSKEQQCVIMPENSKSSIVFRMRDVFLFEKRIYLSECKTGNGKTYRGTMAKTKNDVACQKWSDNSPHKPN
3   63   ------AFQYHSKEQQCVIMAENRKSSIIIRMRDVVLFEKKVYLSECKTGNGKNYRGTMSKTKNGITCQKWSSTSPHRPR
4   63   ------AFQYHSKEQQCVIMAENRKSSIIIRMRDVVLFEKKVYLSECKTGNGKNYRGTMSKTKNGITCQKWSSTSPHRPR
5   53   ------AFQYHSKEQQCVIMAENRKSSIIIRMRDVVLFEKKVYLSECKTGNGKNYRGTMSKTKNGITCQKWSSTSPHRPR
6   63   ------SFQYHSKEQQCVIMAENRKSSIVFRMRDVVLFEKKVYLSECKTGNGKNYRGTMSKTRTGITCQKWSSTSPHRPT
7   63   ------AFQYHSKEQQCVIMAENRKSSIIIRMRDVVLFEKKVYLSECKTGNGKNYRGTMSKTKNGITCQKWSSTSPHRPR
8   63   ------AFQYHSKDQQCVVMAENSKTSPIARMRDVVLFEKRIYLSECKTGNGKNYRGTTSKTKSGVICQKWSVSSPHIPK
9   70   ------AFQYHSKEQQCVVMAENSKNTPVFRMRDVILYEKRIYLLECKTGNGQTYRGTTAETKSGVTCQKWSATSPHVPK
10  63   ------AFQYHSKEPRCVLLAENRKSSPVMRMRDVILFEKRIYLSECKTGTGRSYRGTTSKTKNGVSCQKWSDTSPHIPK
11  63   ------SFQYHSKEQQCVIMAENSKTSSIIRMRDVILFEKRVYLSECKTGIGNSYRGTMSRTKSGVACQKWGATFPHVPN
12  63   ------SFQYHSKEQQCVIMAENSKTSSIIRMRDVILFEKRVYLSECKTGIGKGYRGTMSKTKTGVTCQKWSDTSPHVPK
13  63   ------SFQYHSKEQQCVIMAENSKSTPVLRMRDVILFEKKMYLSECKVGNGKYYRGTVSKTKTGLTCQKWSAETPHKPR
14  63   ------SFQYHSKEQQCVVMAENSKSSAIIRRRDVVLFEKRMYLSECKIGNGRSYRGTKSKTKTGFTCQKWSSSYPHKPN
15  74   CNSDGKAFQYHSKEQQCVIMAENRKSSIIIRMRDVVLFEKKVYLSECKTGNGKNYRGTMSKTKNGITCQKWSSTSPHRPR
16  5    ------SFEYHSKEQQCAIMAENSKSSAVFRMRDVILFQKRIYLSECKTGNGKTYRGTMSKTKNGVACQKWSDTFPHKPN
```

FIGURE 10/3

```
           121       131       141       151       161       171       181       191
           |         |         |         |         |         |         |         |
1   137  FSPATHPSEGLEENYCRNPDNDPQGPWCYTTDPEKRYDYCDILECEEECMHCSGENYDGKISKTMSGLECQAWDSQSPHA
2   137  YTPEKHPLEGLEENYCRNPDNDENGPWCYTTNPDVRFDYCNIPECEEECMHCSGENYEGKISKTKSGLECQAWNSQTPHA
3   137  FSPATHPSEGLEENYCRNPDNDPQGPWCYTTDPEKRYDYCDILECEEECMHCSGENYDGKISKTMSGLECQAWDSQSPHA
4   137  FSPATHPSEGLEENYCRNPDNDPQGPWCYTTDPEKRYDYCDILECEEECMHCSGENYDGKISKTMSGLECQAWDSQSPHA
5   127  FSPATHPSEGLEENYCRNPDNDPQGPWCYTTDPEKRYDYCDILECEEECMHCSGENYDGKISKTMSGLECQAWDSQSPHA
6   137  FSPATHPSEGLEENYCRNPDNDGQGPWCYTTDPEERFDYCDIPECEDECMHCSGENYDGKISKTMSGLECQAWDSQSPHA
7   137  FSPATHPSEGLEENYCRNPDNDAQGPWCYTTDPEHRYDYCDIPECEEACMHCSGENYDGKISKTMSGLECQAWDSQSPHA
8   137  YSPEKFPLAGLEENYCRNPDNDEKGPWCYTTDPETRFDYCDIPECEDECMHCSGEHYEGKISKTMSGIECQSWGSQSPHA
9   144  FSPEKFPLAGLEENYCRNPDNDENGPWCYTTDPDKRYDYCDIPECEDKCMHCSGENYEGKIAKTMSGRDCQAWDSQSPHA
10  137  YSPDKNPSEGLEENYCRNPDNDEKGPWCYTTDPGTRFDYCDIPECEDECMHCSGENYEGKISKTISGLECQPWASQSPHA
11  137  YSPSTHPNEGLEENYCRNPDNDEQGPWCYTTDPDKRYDYCNIPECEEECMYCSGEKYEGKISKTMSGLDCQAWDSQSPHA
12  137  YSPSTHPSEGLEENYCRNPDNDEQGPWCYTTDPDQRYEYCNIPECEEECMYCSGEKYEGKISKTMSGLDCQSWDSQSPHA
13  137  FSPDENPSEGLDQNYCRNPDNDPKGPWCYTMDPEVRYEYCEIIQCEDECMHCSGQNYVGKISRTMSGLECQPWDSQIPHP
14  137  FTPKKYPAEGLEENYCRNPDNDEQGPWCYTTNPDERFDYCDIPECEDECMHCSGENYEGKISKTMSGIECQAWDSQSPHA
15  154  FSPATHPSEGLEENYCRNPDNDPQGPWCYTTDPEKRYDYCDILECEEECMHCSGENYDGKISKTMSGLECQAWDSQSPHA
16  79   YTPEKHPLEGLEENYCRNPDNDEKGPWCYTTDPNQRFDYCSIPQCEDECMHCSGENYEGKVSKTKSGLECQAWNSQTPHA
```

FIGURE 10/4

```
               201       211       221       231       241       251       261       271
                 |         |         |         |         |         |         |         |
1    217   HGYIPSKFPNKNLKKNYCRNPDRELRPWCFTTDPNKRWELCDIPRCTTPPPSSGPTYQCLKGTGENYRGNVAVTVSGHTC
2    217   HGYIPSKFPSKNLKMNYCRNPDGEPRPWCFTMDPNKRWEFCDIPRCTTPPPPSGPTYQCLKGRGESYRGKVSVTVSGHTC
3    217   HGYIPSKFPNKNLKKNYCRNPDGELRPWCFTTDPNKRWELCDIPRCTTPPPSSGPTYQCLKGTGENYRGNVAVTVSGHTC
4    217   HGYIPSKFPNKNLKKNYCRNPDGELRPWCFTTDPNKRWELCDIPRCTTPPPSSGPTYQCLKGTGENYRGNVAVTVSGHTC
5    207   HGYIPSKFPNKNLKKNYCRNPDGELRPWCFTTDPNKRWELCDIPRCTTPPPSSGPTYQCLKGTGENYRGNVAVTVSGHTC
6    217   HGYIPSKFPNKNLKKNYCRNPDGEPRPWCFTTDPNKRWELCDIPRCTTPPPSSGPTYQCLKGTGENYRGDVAVTVSGHTC
7    217   HGYIPSKFPNKNLKKNYCRNPDGEPRPWCFTTDPNKRWELCDIPRCTTPPPSSGPTYQCLKGTGENYRGNVAVTVSGHTC
8    217   HGYLPSKFPNKNLKMNYCRNPDGEPRPWCFTTDPNKRWEFCDIPRCTTPPPTSGPTYQCLKGRGENYRGTVSVTASGHTC
9    224   HGYIPSKFPSKNLKMNYCRNPDGEPRPWCFTTDPQKRWEFCDIPRCTTPPPSSGPKYQCLKGTGKNYGGTVAVTESGHTC
10   217   HGYIPSKFPNKNLRMNYCRNPDGEPRPWCFTMDPDKRWEFCDIPRCSTPPPSSGPTYQCLKGRGENYRGRVSVTQSGLTC
11   217   HGYIPAKFPSKNLKMNYCRNPDGEPRPWCFTTDPTKRWEYCDIPRCTTPPPPPSPTYQCLKGRGENYRGTVSVTVSGKTC
12   217   HGYIPAKFPSKNLKMNYCRNPDGEPRPWCFTTDPNKRWEYCDIPRCTTPPPPPGPTYQCLKGRGENYRGTVSVTASGKTC
13   217   HGFIPSKFPSKNLKMNYCRNPDGEPRPWCFTMDRNKRWEYCDIPRCTTPPPPSGPTYQCLMGNGEHYQGNVAVTVSGLTC
14   217   HGYIPSKFPNKNLKKNYCRNPDGEPRPWCFTMDPKKRWELCDIPRCTTPPPPSGPTHQCLKGRGESYRGKVARTKSGLTC
15   234   HGYIPSKFPNKNLKKNYCRNPDGELRPWCFTTDPNKRWELCDIPRCTTPPPSSGPTYQCLKGTGENYRGNVAVTVSGHTC
16   159   HGYIPSKFPNKNLKMNYCRNPDGEPRPWCFTMDPNKRWEFCDIPRCTTPPPPSGPTYQCLKGKGENYRGKVSVTASGHTC
```

FIGURE 10/5

```
            281       291       301       311       321       331       341         351
              |         |         |         |         |         |         |           |
1     297   QHWSAQTPHTHNRTPENFPCKNLDENYCRNPDGKRAPWCHTTNSQVRWEYCKIPSCDSSPVSTEQLAPTA--PPE-LTPV
2     297   QHWSEQTPHKHNRTPENFPCKNLDENYCRNPDGETAPWCYTTNSEVRWEHCQIPSCESSPITTEYLDAPASVPPE-QTPV
3     297   QHWSAQTPHTHNRTPENFPCKNLDENYCRNPDGKRAPWCHTTNSQVRWEYCKIPSCDSSLVSTEQLAPTA--PPE-LTPV
4     297   QHWSAQTPHTHNRTPENFPCKNLDENYCRNPDGKRAPWCHTTNSQVRWEYCKIPSCDSSLVSTEQLAPTA--PPE-LTPV
5     287   QHWSAQTPHTHNRTPENFPCKNLDENYCRNPDGKRAPWCHTTNSQVRWEYCKIPSCDSSLVSTEQLAPTA--PPE-LTPV
6     297   HGWSAQTPHTHNRTPENFPCKNLDENYCRNPDGEKAPWCYTTNSQVRWEYCKIPSCESSPVSTEPLDPTA--PPE-LTPV
7     297   QRWSAQTPQTHNRTPENFPCKNLDENYCRNPDGEKAPWCYTTNSQVRWEYCKIPSCGSSPVSTEQLDPTA--PPE-LTPV
8     297   QRWSAQSPHKHNRTPENFPCKNLEENYCRNPDGETAPWCYTTDSEVRWDYCKIPSCGSSTTSTEYLDAPV--PPE-QTPV
9     304   QRWSEQTPHKHNRTPENFPCKNLEENYCRNPNGEKAPWCYTTNSKVRWEYCTIPSCESSPLSTERMDVPV--PPE-QTPV
10    297   QRWSEQTPHKHNRTPDNFPCKNLDENYCRNPDGETAPWCYTTSSETRWEYCNIPSCTSSSVPTEITDASE--PPE-QTPV
11    297   QRWSEQTPHRHNRTPENFPCKNLEENYCRNPDGETAPWCYTTDSQLRWEYCEIPSCESSASPDQ---SDSSVPPEEQTPV
12    297   QRWSEQTPHRHNRTPENFPCKNLEENYCRNPDGETAPWCYTTDSQLRWEYCEIPSCGSSVSPDQ---SDSSVLPE-QTPV
13    297   QRWGEQSPHRHDRTPENYPCKNLDENYCRNPDGEPAPWCFTTNSSVRWEFCKIPDCVSSASETEHSDAPVIVPPE-QTPV
14    297   QRWSEQTPHLHNRTPENFPCKDLDENYCRNPDGESAPWCYTTDSKVRWEHCDIPSCASSPTSVEPLDAPA--PPE-ETPV
15    314   QHWSAQTPHTHNRTPENFPCKNLDENYCRNPDGKRAPWCHTTNSQVRWEYCKIPSCDSSLVSTEQLAPTA--PPE-LTPV
16    239   QRWSEQTPHKHNRTPENFPCKNLDENYCRNPDGESAPWCYTTDSEVRWEHCSIPSCESSPLTLDSLDTPASIPPE-QTPV
```

FIGURE 10/6

```
             361       371       381       391       401       411       421       431
               |         |         |         |         |         |         |         |
1    374   VQDCYHGDGQSYRGTSSTTTTGKKCQSWSSMTPHRHQKTPENYPNAGLTMNYCRNPDAD-KGPWCFTTDPSVRWEYCNLK
2    376   VQECYHGNGQSYRGTSSTTITGRKCQSWSSMTPHRHEKTPEHFPEAGLTMNYCRNPDAD-KSPWCYTTDPSVRWEFCNLR
3    374   VQDCYHGDGQSYRGTSSTTTTGKKCQSWSSMTPHRHQKTPENYPNAGLTMNYCRNPDAD-KGPWCFTTDPSVRWEYCNLK
4    374   VQDCYHGDGQSYRGTSSTTTTGKKCQSWSSMTPHRHQKTPENYPNAGLTMNYCRNPDAD-KGPWCFTTDPSVRWEYCNLK
5    364   VQDCYHGDGQSYRGTSSTTTTGKKCQSWSSMTPHRHQKTPENYPNAGLTMNYCRNPDAD-KGPWCFTTDPSVRWEYCNLK
6    374   VQECYHGDGQSYRGTSSTTTTGKKCQSWSSMTPHWHEKTPENFPNAGLTMNYCRNPDAD-KGPWCFTTDPSVRWEYCNLK
7    374   VQDCYHGDGQSYRGTSSTTTTGKKCQSWSSMTPHWHQKTPENYPDAGLTMNYCRNPDAD-KGPWCFTTDPSVRWEYCNLK
8    374   AQDCYRGNGESYRGTSSTTITGRKCQSWVSMTPHRHEKTPGNFPNAGLTMNYCRNPDAD-KSPWCYTTDPRVRWEYCNLK
9    381   PQDCYHGNGQSYRGTSSTTITGRKCQSWSSMTPHRHLKTPENYPNAGLTMNYCRNPDAD-KSPWCYTTDPRVRWEFCNLK
10   374   VQDCYQDKGESYRGTSSITVTGKKCQSWSSMTPHWHQKTPEKYPNADLTMNYCRNPDGD-KGPWCYTTDPSVRWEFCNLR
11   374   VQECYQSDGQSYRGTSSTTITGKKCQSWAAMFPHRHSKTPENFPDAGLEMNYCRNPDGD-KGPWCYTTDPSVRWEYCNLK
12   373   VQECYQGNGKSYRGTSSTTNTGKKCQSWVSMTPHSHSKTPANFPDAGLEMNYCRNPDNDQRGPWCFTTDPSVRWEYCNLK
13   376   VQECYQGNGQSYRGTSSTTITGKKCQPWTSMRPHRHSKTPENYPDADLTMNYCRNPDGD-KGPWCYTTDPSVRWEFCNLK
14   374   VQECYQGNGQSYRGTSSTTITGRKCQSWLSMTPHRHQRTPQNYPNADLTMNYCRNPDDD-IRPWCYTTDPSVRWEYCNLR
15   391   VQDCYHGDGQSYRGTSSTTTTGKKCQSWSSMTPHRHQKTPENYPNAGLTMNYCRNPDAD-KGPWCFTTDPSVRWEYCNLK
16   318   VQECYQGNGQTYRGTSSTTITGKKCQPWSSMSPHRHEKTPERFPNAGLTMNYCRNPDGD-KSPWCYTTDPSVRWEFCNLK
```

FIGURE 10/7

```
                441       451       461       471       481       491       501       511
                  |         |         |         |         |         |         |         |
1   453  KCSGTEASVVA-PPPVVLLPDVETPSEEDCMFGNGKGYRGKRATTVTGTPCQDWAAQEPHRHSIFTPETNPRA-GLEKNY
2   455  KCLDPEASATN-SPAVPQVPSGQEPSASDCMFGNGKGYRGKKATTVMGIPCQEWAAQEPHRHSIFTPETNPQA-GLEKNY
3   453  KCSGTEASVVA-PPPVVQLPNVETPSEEDCMFGNGKGYRGKRATTVTGTPCQDWAAQEPHRHSIFTPETNPRA-GLEKNY
4   453  KCSGTEASVVA-PPPVVQLPNVETPSEEDCMFGNGKGYRGKRATTVTGTPCQDWAAQEPHRHSIFTPETNPRA-GLEKNY
5   443  KCSGTEASVVA-PPPVVQLPNVETPSEEDCMFGNGKGYRGKRATTVTGTPCQDWAAQEPHRHSIFTPETNPRA-GLEKNY
6   453  KCSGTEGSVAA-PPPVAQLPDAETPSEEDCMFGNGKGYRGKKATTVTGTPCQEWAAQEPHSHRIFTPETNPRA-GLEKNY
7   453  KCSGTEGSVVA-PPPVVQLPNVETPSEEDCMFGNGKGYRGKRATTVTGTPCQEWAAQEPHRHSIFTPQTNPRA-GLEKNY
8   453  KCSETEQQVTN-FPAIAQVPSVEDLSE-DCMFGNGKRYRGKRATTVAGVPCQEWAAQEPHRHSIFTPETNPRA-GLEKNY
9   460  KCSETPEQV----PAAPQAPGVENPPEADCMIGMGKSYRGKKATTVAGVPCQEWAAQEPHHHSIFTPETNPQS-GLERNY
10  453  RCSETQQSFSNSSPTDTQVPSVQEPSEPDCMLGIGKGYQGKKATTVTGTRCQAWAAQEPHRHSIFTPEANPWA-NLEKNY
11  453  RCSETGGSVVE-LPTVSQEPSGPSDSETDCMYGNGKDYRGKTAVTAAGTPCQGWAAQEPHRHSIFTPQTNPRA-GLEKNY
12  453  RCSETGGGVAE-SAIVPQVPSAPGTSETDCMYGNGKEYRGKTAVTAAGTPCQEWAAQEPHSHRIFTPQTNPRA-GLEKNY
13  455  KCSGTEMSATN-SSPV-QVSSASESSEQDCIIDNGKGYRGTKATTGAGTPCQAWAAQEPHRHSIFTPETNPRA-DLQENY
14  453  RCSEPAASPAA-TVPTAQLPRPEATFEPDCMFGNGKGYRGKKATTADGTPCQGWAAQEPHRHNIFTPETNPRA-GLERNY
15  470  KCSGTEASVVA-PPPVVQLPNVETPSEEDCMFGNGKGYRGKRATTVTGTPCQDWAAQEPHRHSIFTPETNPRA-GLEKNY
16  397  KCLDTEESGTS-SPTVPQVPSGEEPSETDCMFGNGKGYRGKKATTVLGIPCQEWTAQEPHKHSIFTPETNPRAEHLLCPT
17  1    -------------------------IRLDCMFGNGKRYRGKKATTVTGTPCQEWAAKEPHSHLIFTPETYPRA-GLEKNY
18  1    ---------------APQAPSVENPPEADCMLGIGKGYRGKKATTVAGVPCQEWAAQEPHRHGIFTPETNPRA-GLEKNY
```

FIGURE 10/8

```
                                       521       531       541        551       561       571
                                         |         |         |          |         |        |
1   531  CRNPDG--------------DVGGPWCYTTNPRKLYDYCDVPQCAA-PSFDCGKPQVEPKKCPGRVVGGCVAHPHSWPWQ
2   533  CRNPDG--------------DVNGPWCYTMNQRKLFDYCDVPQCVS-TSFDCGKPQVEPKKCPGRVVGGCVANPHSWPWQ
3   531  CRNPDG--------------DVGGPWCYTTNPRKLYDYCDVPQCAS-PSFDCGKPQVEPKKCPGRVVGGCVAHPHSWPWQ
4   531  CRNPDG--------------DVGGPWCYTTNPRKLYDYCDVPQCAS-PSFDCGKPQVEPKKCPGRVVGGCVAHPHSWPWQ
5   521  CRNPDG--------------DVGGPWCYTTNPRKLYDYCDVPQCAS-PSFDCGKPQVEPKKCPGRVVGGCVAHPHSWPWQ
6   531  CRNPDG--------------DVGGPWCYTTNPRKLFDYCDVPQCAA-SSFDCGKPQVEPKKCPGRVVGGCVAYPHSWPWQ
7   531  CRNPDG--------------DEGGPWCYTTNPRKHYDYCDVPQCAS-SSFDCGKPQVEPKKCPGRVVGGCVANAHSWPWQ
8   530  CRNPDG--------------DDNGPWCYTTNPQKLFDYCDVPQCVT-SSFDCGKPKVEPKKCPARVVGGCVSIPHSWPWQ
9   535  CRNPDG--------------DVNGPWCYTMNPRKLFDYCDVPQC-E-SSFDCGKPKVEPKKCSGRIVGGCVSKPHSWPWQ
10  532  CRNPDG--------------DVNGPWCYTMNPQKLFDYCDVPQCES-SPFDCGKPKVEPKKCSGRIVGGCVAIAHSWPWQ
11  531  CRNPDG--------------DVNGPWCYTTNPRKLYDYCDIPLCASASSFECGKPQVEPKKCPGRVVGGCVANPHSWPWQ
12  531  CRNPDG--------------DVNGPWCYTMNPRKLYDYCNIPLCASLSSFECGKPQVEPKKCPGRVVGGCVANPHSWPWQ
13  532  CRNPDG--------------DANGPWCYTTNPRKLFDYCDIPHCVSPSSADCGKPKVEPKKCPGRVVGGCVANPHSWPWQ
14  531  CRNPDG--------------DTNGPWCYTMNPRKLYDYCDVPQCASSSSYDCGKPKVEPKKCPGRVVGGCVANPHSWPWQ
15  548  CRNPDG--------------DVGGPWCYTTNPRKLYDYCDVPQCAS-PSFDCGKPQVEPKKCPGRVVGGCVAHPHSWPWQ
16  476  CLVPSVPTVFFFFFFFFLFLDVNGPWCYTTNPRKLFDYCDIPQCAS-GSFDCGKPQVEPKKCPGRVVGGCVANPHSWPWQ
17  55   CRNPDG--------------DVGGPWCYTTNPRKLYDYCDVPQCAS-SSFDCGKPQVEPKKCPGRVVGGCVAHAHSWPWQ
18  65   CRNPDG--------------DVNGPWCYTTNPRKLFDYCDIPQC-E-SSFDCGKPKVEPKKCPARVVGGCVATPHSWPWQ
```

FIGURE 10/9

```
              581                 591        601        611        621          631        641
               |                   |          |          |          |            |          |
1   596  VSLRTRF-GM-----HFCGGTLISPEWVLTAAHCLEKSPRPSSYKVILGAHQEVNLEPHVQEIEVSRLFLEPTRKDIALL
2   598  ISLRTRY-GK-----HFCGGTLISPEWVLTAAHCLERSSRPASYKVILGAHKEVNLESDVQEIEVYKLFLEPTRADIALL
3   596  VSLRTRL-GM-----HFCGGTLISPEWVLTAAHCLEKSPRPSSYKVILGAHQEVKLEPHVQEIEVSRLFLEPTRTDIALL
4   596  VSLRTSS-NIAGKYWHFCGGTLISPEWVLTAAHCLEKSPRPSSYKVILGAHQEVKLEPHVQEIEVSRLFLEPTRTDIALL
5   586  VSLRTRL-GM-----HFCGGTLISPEWVLTAAHCLEKSPRPSSYKVILGAHQEVKLEPHVQEIEVSRLFLEPTRTDIALL
6   596  ISLRTRL-GM-----HFCGGTLISPEWVLTAAHCLEKSSRPSFYKVILGAHREVHLEPHVQEIEVSKMFSEPARADIALL
7   596  VSLRTRF-GT-----HFCGGTLISPEWVLTAAHCLEKSPRPSSYKVILGAHQEVNLEPHVQEIEVSRLFLEPTRADIALL
8   595  ISLRHRY-GG-----HFCGGTLISPEWVLTAKHCLEKSSSPSSYKVILGAHEEYHLGEGVQEIDVSKLFKEPSEADIALL
9   599  VSLR-RS-SR-----HFCGGTLISPKWVLTAAHCLDNILALSFYKVILGAHNEKVREQSVQEIPVSRLFREPSQADIALL
10  597  ISLRTRF-GR-----HFCGGTLISPEWVLTAAHCLERSSRPSTYKVVLGTHHELRLAAGAQQIDVSKLFLEPSRADIALL
11  597  ISLRTRFTGQ-----HFCGGTLIAPEWVLTAAHCLEKSSRPEFYKVILGAHEEYIRGSDVQEISVAKLILEPNNRDIALL
12  597  ISLRTRFSGQ-----HFCGGTLISPEWVLTAAHCLEKSSRPEFYKVILGAHEERILGSDVQQIAVTKLVLEPNDADIALL
13  598  VSLR-RF-GQ-----HFCGGTLISPEWVVTAAHCLEKFSNPAIYKVVLGAHQETRLERDVQIKGVTKMFLEPYRADIALL
14  597  ISLRTRT-GQ-----HFCGGTLIAPEWVLTAAHCLEKYPRPSAYRVILGAHKEVNLELDVQDIDVAKLFLEPSRADIALM
15  613  VSLRTRL-GM-----HFCGGTLISPEWVLTAAHCLEKSPRPSSYKVILGAHQEVKLEPHVQEIEVSRLFLEPTRTDIALL
16  555  ISLRTRF-GQ-----HFCGGTLISPEWVLTAAHCLERSPRPAAYKVILGAHREFNLESDVQEIEVSKLFLEPTHADIALI
17  120  VSLRTRF-GM-----HFCGGTLISPEWVLTAAHCLEKSPRPSFYKVILGAHQEVRLEPHVQEIEVSKMFSEPAGADIALL
18  129  VSLRRRS-RE-----HFCGGTLISPEWVLTAAHCLDSILGPSFYTVILGAHYEMAREASVQEIPVSRLFLEPSRADIALL
```

FIGURE 10/10

```
              651       661       671       681       691       701       711       721
               |         |         |         |         |         |         |         |
1    670   KLSSPAVITDKVIPACLPSPNYVVADRTECFITGWGETQGTFGAGLLKEAQLPVIENKVCNRYEFLNGRVQSTEL
2    672   KLSSPAVITSKVIPACLPPPNYVVADRTLCYITGWGETQGTYGAGLLKEAQLPVIENKVCNRYEYLNGRVKSTEL
3    670   KLSSPAIITDKVIPACLPSPNYVVADRTECFITGWGETQGTFGAGLLKEAQLPVIENKVCNRNEFLNGRVKSTEL
4    675   KLSSPAIITDKVIPACLPSPNYVVADRTECFITGWGETQGTFGAGLLKEAQLPVIENKVCNRNEFLNGRVKSTEL
5    660   KLSSPAIITDKVIPACLPSPNYVVADRTECFITGWGETQGTFGAGLLKEAQLPVIENKVCNRNEFLNGRVKSTEL
6    670   KLSSPAIITDKVIPACLPSPNYVVADRTECFITGWGETQGTYGAGLLKEARLPVIENKVCNRYEFLNGTVKTTEL
7    670   KLSSPAVITDKVIPACLPSPNYVVAGRTECFITGWGETQGTFGAGLLKEAQLPVIENKVCNRYEFLNGRVKSTEL
8    669   KLSSPAIITDKVIPACLPTPNYVVADRTACYITGWGETKGTYGAGLLKEARLPVIENKVCNRYEYLGGKVSPNEL
9    672   KLSRPAIITKEVIPACLPPPNYMVAARTECYITGWGETQGTFGEGLLKEAHLPVIENKVCNRNEYLDGRVKPTEL
10   671   KLSSPAIITQNVIPACLPPADYVVANWAECFVTGWGETQDSSNAGVLKEAQLPVIENKVCNRYEYLNGRVKSTEL
11   672   KLSRPATITDKVIPACLPSPNYMVADRTICYITGWGETQGTFGAGRLKEAQLPVIENKVCNRVEYLNNRVKSTEL
12   672   KLSRPATITDNVIPACLPSPNYVVADRTLCYITGWGETKGTPGAGRLKEAQLPVIENKVCNRAEYLNNRVKSTEL
13   671   KLSSPAIITDKIIPACLPNSNYMVADRSLCYITGWGETKGTYGAGLLKEAQLPVIENKVCNRQELLNGRVRSTEL
14   671   KLSSL-----------------------------EWAWTYGAGLLKEAQLPVIENKVCNRFEYLNGRVRSTEL
15   687   KLSSPAIITDKVIPACLPSPNYVVADRTECFITGWGETQGTFGAGLLKEAQLPVIENKVCNRNEFLNGRVKSTEL
16   629   KLQSPAVLTSKVIPACLPSPNYVVADRTLCYITGWGETQGTFGVGLLKEAQLPVIENKVCNRYEYLNGKVKSTEL
17   194   KLSSPAIITDKVIPACLPSPNYVVADRTECFITGWGETQGTYGAGLLKEARLPVIENKVCNRYEFLNGRVKSTEL
18   203   KLSSPAVITDEVIPACLPSPNYVVADKTVCYITGWGETQGTFGVGRLKEARLPVIENKVCNRYEYLNGRVKSTEL
```

FIGURE 10/11

		731 741 751 761 771 781 791	
1	745	CAGHLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYVRVSRFVTWIEGVMRNN	810
2	747	CAGNLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYVRVSRFVTWIEGIMRNN	812
3	745	CAGHLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYVRVSRFVTWIEGVMRNN	810
4	750	CAGHLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYVRVSRFVTWIEGVMRNN	815
5	735	CAGHLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYVRVSRFVTWIEGVMRNN	800
6	745	CAGHLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYVRVSRFVTWIEGVMRNN	810
7	745	CAGHLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYVRVSRFVTWIEGVMRNN	810
8	744	CAGHLAGGIDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCALPNKPGVYVRVSRFVTWIEEIMRRN	809
9	747	CAGHLIGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYVRVSPYVPWIEETMRRN	812
10	746	CAGHLVGGVDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYVRVSSFINWIERIMQSN	811
11	747	CAGQLAGGVDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYVRVSRFVDWIEREMRNN	812
12	747	CAGHLAGGIDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYVRVSRYVNWIEREMRND	812
13	746	CAGHLAGGVDSCQGDSGGPLVCFEKDRYILQGVTSWGLGCARPNKPGVYVRVSRYVSWLQDVMRNN	811
14	715	CAGHLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYVRVSRFVDWIERTMRNN	780
15	762	CAGHLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYVRVSRFVTWIEGVMRNN	827
16	704	CAGNLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYVRVSRFVTWIEEIMRNN	769
17	269	CAGHLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYVRVSRFVTWIEGVMRNN	334
18	278	CAGDLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYVRVSTYVPWIEETMRRY	343

FIGURE 10/12

VARIANTS OF PLASMINOGEN AND PLASMIN

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is the National Stage of International Application Serial No. PCT/EP2010/059902, filed on Jul. 9, 2010, which claims the benefit of European Application Serial No. EP09165237.0, filed on Jul. 10, 2009, and U.S. Application Ser. No. 61/224,514, filed on Jul. 10, 2009.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Oct. 21, 2014, is named 35824-0004US1 SL.txt and is 147,827 bytes in size.

FIELD OF THE INVENTION

The invention relates to variants of plasminogen and plasmin comprising one or more point mutations in the catalytic domain which reduce or prevent autocatylic destruction of the protease activity of plasmin. Compositions, uses and methods of using said variants of plasminogen and plasmin are also disclosed.

BACKGROUND TO THE INVENTION

Activation of the zymogen plasminogen results in the formation of the fibrinolytically/thrombolytically active serine proteinase plasmin. Activation of endogenous plasminogen can be triggered or enhanced by the administration of a plasminogen activator such as urokinase, streptokinase, staphylokinase or tPA, or any variant thereof. Upon activation, the plasminogen protein is proteolytically cleaved into a heavy chain comprising the 5 kringle domains and a light chain comprising the catalytic domain. Both chains are held together via 2 disulfide bonds. After activation, an autolytic cleavage removes an N-terminal segment from the heavy chain (78 amino acids of human plasmin; 77 amino acids of bovine plasmin) and the bovine plasmin heavy chain can be further autocatalytically cleaved between kringles 3 and 4, hence giving rise to bovine midiplasmin (Christensen et al. 1995, Biochem J 305, 97-102). Activation of plasminogen to plasmin, triggered by the cleavage of the R561-V562 peptide bond in human plasminogen, induces a large conformational change in the light chain, said change resulting in the priming, or activation, of the catalytic triad within said light chain. Bacterial plasminogen activators such as streptokinase and staphylokinase form a complex with plasminogen and, without cleavage of the R561-V562 peptide bond of plasminogen, the catalytic site of plasminogen is activated due to conformational changes upon activator-plasminogen complex formation (plasminogen activation mechanisms are summarized in, e.g., the Introduction section of Terzyan et al. 2004; Proteins 56: 277-284).

Whereas plasminogen activators act as indirect thrombolytic agents, it has alternatively been suggested to use plasmin itself as a direct fibrinolytic/thrombolytic agent. Such direct use is, however, hampered by the fact that plasmin is, like many proteases, subject to autocatalytic proteolytic degradation which follows second order kinetics subject to product inhibition (Jespersen et al. 1986, Thrombosis Research 41, 395-404).

In the early 1960's it was established that plasmin can be stabilized at acidic pH, or alternatively at neutral pH provided an amino acid such as lysine is present. Nevertheless, autolytic cleavage after Lys104, Arg189 and Lys622 (numbering relative to Lys-plasmin) were reported even when plasmin is stored at pH 3.8 (WO01/36608). When plasmin is stored at the even lower pH of 2.2, non-autolytic acid cleavage occurs between Asp-Pro (D-P) at positions Asp62, Asp154 and Asp346 (WO01/36608). This illustrates that pH can be lowered to a point where no apparent autocatylic degradation occurs anymore but at which acid hydrolysis is becoming a factor of destabilization. No information is present in WO01/36608 as to which peptide bonds in plasmin are vulnerable to (autocatalytic) hydrolysis at neutral pH. Known stabilizers of plasmin include glycerol, sufficiently high ionic strength, fibrinogen and ϵ-aminocaproic acid (EACA), as disclosed by Jespersen et al. (1986, Thromb Res 41, 395-404). Lysine and lysine-derivatives (such as EACA and tranexamic acid) and p-aminomethylbenzoic acid (PAMBA) are some further known stabilizers (Uehsima et al. 1996, Clin Chim Acta 245, 7-18; Verstraete 1985, Drugs 29, 236-261). U.S. Pat. No. 4,462,980 reported on the formation of plasmin aggregates contributing to plasmin degradation despite storage at acidic conditions. A solution to this problem was provided in U.S. Pat. No. 4,462,980 by means of adding a polyhydroxy compound. Other ways of stabilizing plasmin include the addition of oligopeptidic compounds (e.g. U.S. Pat. No. 5,879,923). Alternatively, the catalytic site of plasmin can be reversibly blocked by means of derivatization, e.g. acylation (EP 0009879). Pegylation of plasmin has also been suggested as a means to stabilize the enzyme (WO 93/15189).

A number of plasmin variants other than truncated forms of plasmin have been described and include a chimeric microplasmin (WO 2004/045558) and variants with a point mutation at the two-chain cleavage site (U.S. Pat. No. 5,087,572) or at a catalytic triad amino acid (Mhashilkar et al. 1993, Proc Natl Acad Sci USA 90, 5374-5377; Wang et al., 2001, J Mol Biol 295, 903-914). Wang et al. (1995, Protein Science 4, 1758-1767 and 1768-1779) reported an extensive series of microplasminogen mutants at amino acid positions 545, 548, 550, 555, 556, 558, 560-564, 585, 740 and 788. A double mutant wherein cysteines at amino acid positions 558 and 566 were substituted for serines was reported by Linde et al. (1998, Eur J Biochem 251, 472-479). Takeda-Shitaka et al. (1999, Chem Pharm Bull 47, 322-328) refer to a plasmin variant with reduced activity, the variation involving the substitution of alanine at amino acid position 601 to threonine. All amino acid positions referred to above are relative to Glu-plasminogen starting with Glu at amino acid position 1. A non-cleavable plasminogen variant (cleavage between heavy and light chain impaired) is described in WO 91/08297. Dawson et al. (1994, Biochemistry 33, 12042-12047) describe the reduced affinity for streptokinase of a Glu-plasminogen variant with a Glu instead of Arg at position 719 (R719E). Jespers et al. (1998, Biochemistry 37, 6380-6386) produced in an Ala-scan the series of phage-displayed microplasminogen single-site mutants H569A, R610A, K615A, D660A, Y672A, R712A, R719A, T782A, R789A, and found that arginine at position 719 is key for interaction with staphylokinase; the D660A mutant was not further characterized due to very low expression; only the R719A mutant was additionally produced in soluble form. None of the mutants showed a gross change in proteolytic activity (substrate S-2403). Jespers et al. (1998) also included an active site mutant S741A in their analysis; the crystal structure of this mutant is disclosed in Wang et al. (2000, J Mol Biol 295, 903-914). In further attempts to unravel the streptokinase/

plasminogen interaction sites, Terzyan et al. (2004, Proteins 56, 277-284) reported a number of microplasminogen mutants (K698M, D740N, S741A) in an already mutated background (R561A), the latter prohibiting proteolytic activation of plasminogen and thus prohibiting formation of active microplasmin (which would complicate the study of the contact-activation mechanism of the streptokinase-microplasminogen complex). Terzyan et al. (2004) further mention an "inadvertent" triple mutant R561A/H569Y/K698M apparently functionally indifferent from the double mutant R561A/K698M. Wang et al. (2000, Eur J Biochem 267, 3994-4001), in studying streptokinase/plasmin(ogen) interaction, produced a set of microplasminogen (amino acids 530-791 of Glu-plasminogen) mutants in a Cys536Ala and Cys541 Ser background. These mutants include the R561A mutation as described above (Terzyan et al. (2004)) as well as R561A/K698G, R561A/K698A and R561A/K698Q double mutants. In the same C536A/C541S background, single K698G and K698A mutations were introduced also, of which the K698G was not characterized further due to difficulties with purification. The above studies aimed at obtaining a better understanding of the characteristics of the plasminogen/plasmin molecule and did not report any clinical usefulness or benefit or putative clinical advantages of the plasminogen/plasmin mutants. Peisach et al. (1999, Biochemistry 38, 11180-11188) succeeded in determining the crystal structure of microplasminogen containing the M585Q, V673M and M788L mutations.

Nguyen & Chrambach (1981, Preparative Biochem 11, 159-172) reported the presence of "a minor and unidentified protein component" of 10.0 kDa based on reducing SDS-PAGE of a crude commercial preparation of urokinase-activated plasmin (Homolysin). The differences in autolysis of human plasmin depending on pH have been described in detail by Shi & Wu (1988, Thrombosis Research 51, 355-364). Ohyama et al. (2004, Eur J Biochem 271, 809-820) proposed the use of non-lysine analog plasminogen modulators in treatment of cancer due to the enhancement of plasmin autoproteolysis by such compounds which results in the enhanced formation of angiostatins (in the presence of the plasminogen activator urokinase). Table 3 of Ohyama et al. (2004) lists as many as 15 cleavage sites within plasmin subjected to autoproteolyis-enhancing compounds. In discussing their observations in view of prior investigations, it would seem that the autoproteolyis-enhancing compounds are more or less selectively enhancing proteolysis of the B/light-chain whereas minimum degradation of both A/heavy- and B-chain was found in the absence of the autoproteolyis-enhancing compounds.

It is clear that none of the above methods/variants solves the problem of providing a plasmin stabilized at the molecular level. The provision of a plasmin variant (or of a corresponding plasminogen variant from which plasmin can be derived) with a catalytic domain intrinsically resistant to autocatalytic degradation would be a significant step forward towards efficient and safe long-term storage as well as towards efficient and safe therapeutic use of plasmin such as in thrombolytic therapy or in the induction of posterior vitreous detachment or vitreous liquefaction in the eye.

SUMMARY OF THE INVENTION

The current invention relates to isolated plasminogen variants or plasmins obtained from it, or to isolated plasmin variants, or to proteolytically active or reversible inactive derivatives of any of said plasmins characterized in that said plasminogen or plasmin variants or said derivatives comprise in their catalytic domain the mutation of at least one internal amino acid at position P of which the peptide bond with internal amino acid at position P+1 is prone to autoproteolysis into an amino acid of which the peptide bond with internal amino acid at position P+1 is less or not prone to autoproteolysis.

Alternatively, the plasminogen variant, plasmin variant, or plasmin derivative according to the invention comprises in its catalytic domain the mutation of at least two internal amino acids at positions P and P' of which the peptide bond with internal amino acids at positions P+1 and P'+1 are prone to autoproteolysis into amino acids of which the peptide bond with internal amino acids at positions P+1 and P'+1 is less or not prone to autoproteolysis.

In particular, said internal amino acids at positions P or P and P' are lysines or arginines.

More specifically, said at least one or two internal amino acids at position P or at positions P and P' may be at least one or at least two of:

(i) lysine at position 137 of the human plasmin catalytic domain, or the corresponding lysine or arginine of a non-human plasmin catalytic domain;
(ii) lysine at position 147 of the human plasmin catalytic domain, or the corresponding lysine or arginine of a non-human plasmin catalytic domain; or
(iii) arginine at position 158 of the human plasmin catalytic domain, or the corresponding arginine or lysine of a non-human plasmin catalytic domain;

wherein said human plasmin catalytic domain is starting with the amino acid valine at position 1 which is the same valine amino acid occurring at position 562 of human Glu-plasminogen.

Alternatively, said at least one internal amino acid at position P is the lysine at position 147 of the human plasmin catalytic domain, or is the corresponding lysine or arginine of a non-human plasmin catalytic domain, wherein said human plasmin catalytic domain is starting with the amino acid valine at position 1 which is the same valine amino acid occurring at position 562 of human Glu-plasminogen. Optionally, the plasminogen variants, plasmin variants, or plasmin derivatives with a mutation of the lysine at position 147 of the human plasmin catalytic domain (or corresponding lysine or arginine of a non-human plasmin catalytic domain) may further comprise a mutation of the internal amino acids at positions 137 and/or 158 of the human catalytic domain or of the corresponding lysines and/or arginines of a non-human plasmin catalytic domain, wherein said human plasmin catalytic domain is starting with the amino acid valine at position 1 which is the same valine amino acid occurring at position 562 of human Glu-plasminogen.

In a further alternative, the plasminogen variants, plasmin variants, or plasmin derivatives according to the invention are such that:

(i) if the mutation of said at least one internal amino acid at position P is the mutation of the lysine at position 137 of the human plasmin catalytic domain (which is amino acid position 698 relative to human Glu-plasminogen) into an amino acid rendering the peptide bond between amino acids 137 and 138 more resistant to autoproteolysis, said plasminogen variant, plasmin variant or plasmin derivative comprises an intact activation site at amino acid positions 561 and 562 (relative to human Glu-plasminogen), and, when amino acids at position 536 and 541 (relative to human Glu-plasminogen) outside the catalytic domain are present, said amino acids are the wild-type cysteines, or
(ii) if the mutation of said at least one internal amino acid at position P is the mutation of the arginine at position 158 of

the human plasmin catalytic domain (which is amino acid position 719 relative to human Glu-plasminogen) into an alanine or glutamate, then at least one other internal amino acid of the human plasmin catalytic domain at a position P' of which the peptide bond with internal amino acid at position P'+1 is prone to autoproteolysis is mutated into an amino acid of which the peptide bond with internal amino acid at position P'+1 is less or not prone to autoproteolysis.

The plasminogen variant, plasmin variant, or plasmin derivative according to (i) or (ii) above may further comprise a mutation of the internal amino acid at position 147 of the human catalytic domain or of the corresponding lysine or arginine of a non-human plasmin catalytic domain, wherein said human plasmin catalytic domain is starting with the amino acid valine at position 1 which is the same valine amino acid occurring at position 562 of human Glu-plasminogen.

Any of the plasminogen variants, plasmin variants, or plasmin derivatives according to the invention may be characterized further in that its autolysis constant is at most 95% of the autolysis constant of wildtype plasmin.

Any of the plasminogen variants, plasmin variants, or plasmin derivatives according to the invention may be characterized further in that the catalytic constant k_{cat} is in the range of 10% to 200% of the k_{cat} of wildtype plasmin.

Any of the plasminogen variants, plasmin variants, or plasmin derivatives according to the invention may be characterized further in that its autolysis constant is at most 95% of the autolysis constant of wildtype plasmin and its catalytic constant k_{cat} is in the range of 10% to 200% of the k_{cat} of wildtype plasmin.

Without imposing any limitation, any of the above plasminogen variants, plasmin variants, or plasmin derivatives according to the invention may be one of Glu-plasminogen or Glu-plasmin, Lys-plasminogen or Lys-plasmin, midiplasminogen or midiplasmin, miniplasminogen or miniplasmin, microplasminogen or microplasmin, deltaplasminogen or deltaplasmin.

The invention further relates to the isolated plasminogen variants, plasmin variants, or plasmin derivatives according to the invention, or a combination of any thereof for use as a medicament.

The invention also relates to compositions comprising an isolated plasminogen variant, plasmin variant, or plasmin derivative according to the invention, or a combination of any thereof, and at least one of a pharmaceutically acceptable diluent, carrier or adjuvant. Such composition may optionally further comprise at least one of an anticoagulant, a thrombolytic agent, an anti-inflammatory agent, an antiviral agent, an antibacterial agent, an antifungal agent, an anti-angiogenic agent, an anti-mitotic agent, an antihistamine or an anaesthetic.

The invention also includes any beneficial application of an isolated plasminogen variant, plasmin variant, or plasmin derivative according to the invention. Without imposing any limitation, these include: inducing or promoting lysis of a pathological fibrin deposit in a subject, inducing posterior vitreous detachment in the eye and/or for inducing liquefaction of the vitreous in the eye, facilitating surgical vitrectomy in the eye in a subject, enzymatic debridement of injured tissue of a subject, reducing circulating fibrinogen in a subject, reducing α2-antiplasmin levels in a subject, reducing the risk of pathological fibrin deposition.

The invention further relates to methods for screening for an autoproteolytically stable plasmin variant, said methods comprising the steps of:

(i) identifying in the catalytic domain of wild-type plasmin at least one internal amino acid at position P of which the peptide bond with internal amino acid at position P+1 is prone to autoproteolysis,

(ii) mutating the amino acid at position P identified in (i) into an amino acid of which the peptide bond with internal amino acid at position P+1 is less or not prone to autoproteolysis,

(iii) determining the autoproteolytic stability of the mutant obtained from (ii), and

(iv) selecting from (iii) a mutant that is autoproteolytically stable as the autoproteolytically stable variant.

Alternatively, such methods for screening for an autoproteolytically stable plasmin variant may comprise the steps of:

(i) mutating one or more of the arginine or lysine amino acids at positions 137, 147 and 158 of the human plasmin catalytic domain, or of the corresponding arginines or lysines of a non-human plasmin, into an amino acid different from the natural amino acid,

(ii) determining the autoproteolytic stability of the mutant obtained from (i), and

(iii) selecting from (ii) a mutant that is autoproteolytically stable as the autoproteolytically stable plasmin variant;

wherein said human plasmin catalytic domain is starting with the amino acid valine at position which is the same valine amino acid occurring at position 562 of human Glu-plasminogen.

Any of the above screening methods may optionally further comprise a step wherein the proteolytic activity of the autoproteolytically stable plasmin variant is determined.

The invention further includes methods for enhancing long-term storage stability of a plasmin-comprising composition, said methods comprising the step of identifying an autoproteolytically stable plasmin variant capable of being stored over a long time without significant loss of proteolytic activity.

The invention further includes methods for producing a plasminogen variant according to the invention, said methods including the steps of:

(i) introducing a nucleic acid encoding a plasminogen according to the invention in a suitable host cell capable of expressing said plasminogen;

(ii) growing the host cell obtained in (i) under conditions and during a time sufficient for expression of said plasminogen in said host cell; and

(iii) harvesting the plasminogen expressed in (ii).

Such methods may optionally further include a step (iv) wherein the plasminogen harvested in (iii) is purified.

The invention likewise includes methods for producing a plasmin variant according to the invention, said methods including the steps of:

(i) introducing a nucleic acid encoding a plasminogen according to the invention in a suitable host cell capable of expressing said plasminogen;

(ii) growing the host cell obtained in (i) under conditions and during a time sufficient for expression of said plasminogen in said host cell;

(iii) harvesting the plasminogen expressed in (ii);

(iv) activating the plasminogen of (iii) to plasmin.

Such methods may further optionally comprise a step wherein the plasminogen harvested in (iii) is purified prior to activation in (iv). Further, in any method for producing a plasmin variant according to the invention, the active plasmin obtained in (iv) may optionally be purified. Yet further, the active plasmin variant produced according to a method of the invention may optionally be derivatized and/or reversibly inactivated.

The invention further relates to isolated nucleic acid sequences encoding a plasminogen variant or plasmin variant according to the invention. Recombinant vectors comprising such nucleic acids are also part of the invention, as are host cells transformed with such nucleic acid or recombinant vector.

FIGURE LEGENDS

FIG. **1**. Amino acid sequence with double numbering of the amino acid positions of wild-type human Glu-plasminogen (1 to 791) and of the plasmin catalytic domain (1 to 230, amino acid sequence and numbering in bold). Microplasminogen as used for demonstrating the invention starts at amino acid position 543 (numbering relative to Glu-plasminogen). The highlighted amino acids at amino acid positions 137, 147 and 158 (numbering relative to plasmin catalytic domain) were determined to be amino acids of which the peptide bond with amino acids at positions 138, 148 and 159, respectively, are sensitive to autocatalytic cleavage. Kringle domains (as derived from the information included in GenBank accession number AAA36451) are boxed and their amino acid sequences typed alternating in normal and italic letters. The catalytic triad amino acids are circled.

FIG. **2**. Size exclusion chromatography (SEC) profile of large-scale produced microplasmin. The eluates corresponding to fraction number 5 (pre-peak 1), fraction numbers 7&8 (pre-peak 2), fraction numbers 10-12 (microplasmin peak), and fraction numbers 15&16 (post-peak) were collected and the material therein subjected to N-terminal amino acid sequencing (Edman degradation). The peak eluting around fraction numbers 17-18 corresponds to the buffer peak. AU: absorbance units.

FIG. **3**. Reducing SDS-PAGE analysis of large-scale produced microplasmin. Lane 1: molecular weight ladder, with molecular weights indicated at the left. Lane 2: microplasminogen. Lane 3: microplasmin at pH 3.1. Lane 4: microplasmin at pH 4.0. Lane 5: microplasmin at pH 5.0. Lane 6: microplasmin at pH 6.0. Lane 7: microplasmin at pH 7.0. All samples (final protein concentration 0.6 mg/mL) were left for 4 hrs at 20° C. at the indicated pH and then frozen at −70° C. The gel was stained with Coomassie Brilliant Blue. μP1g=microplasminogen, μP1=plasmin, front=leading gel front.

FIG. **4**. Microplasmin was incubated in a neutral-pH buffer, and samples were collected after the indicated times and analyzed by SDS-PAGE (A) or western-blot (B). Arrow "a" indicates the intact microplasmin, whereas arrows "b" and "c" indicate the ~15 kDa and ~10 kDa fragments, respectively, that are autocatalytically produced.

FIG. **5**. The kinetics of microplasmin autolysis as assessed by western-blot (circles) corresponds to the loss of microplasmin activity (squares).

FIG. **6**. (A) Microplasmin was diluted in PBS (squares) or in porcine eye vitreous (circles) to a final concentration of 1.53 μM, and residual concentration of active microplasmin was measured at various time points. (B) Porcine eye vitreous samples were collected at the indicated time points and analyzed by western blot. The arrow indicates a ~15 kDa fragment.

FIG. **7**. (A) Immuno-affinity chromatogram of the microplasmin variant Lys137Met (K137M) on an immobilized anti-microplasmin antibody. Collected elution fractions are numbered 1-11 above the X-axis (elution volume). (B) Reducing SDS-PAGE analysis of elution fractions of immune-affinity performed in (A). Lane 1: molecular weight ladder. Lane 2: eluate fraction 2. Lane 3: eluate fraction 3; Lane 4: eluate fraction 4; Lane 5: eluate fraction 5; Lane 6: eluate fraction 6; Lane 7: crude supernatant. The gel was Coomassie-stained.

FIG. **8**. (A) Activation of the K137M variant with recombinant staphylokinase. Activity reached a maximum after 10 min (indicated by the arrow), then decreased as autolytic inactivation occurred. (B) Reducing SDS-PAGE of the K137M variant indicating that activation with staphylokinase is nearly complete within 10 min, and that loss of activity results from autolytic degradation, as evidenced by the accumulation two fragments of ~17 and ~8 kDa. Lanes 1-7 represent samples collected 0 min, 10 min, 1 h, 2 h, 3 h, 6 h and 24 h after addition of staphylokinase. (▲) Microplasminogen, (▼) microplasmin, (∇) autolytic degradation fragments. (C)HPLC analysis of samples collected 0 min, 10 min and 6 h after addition of staphylokinase. The HPLC profile obtained 10 min after addition of staphylokinase indicates that ~85% of the inactive microplasminogen has been converted into the active microplasmin species, and the HPLC profile at t=6 h shows the presence of the autolytic degradation fragments (∇), in agreement with the SDS-gel showed in (B). The microplasmin peak area at t=10 min (arrow) was used to calculate the concentration of active species by comparison with a standard curve established with highly purified microplasmin (not shown). All HPLC data were obtained using an Acquity HPLC instrument (Waters). The microplasmin samples were typically diluted 5-fold in 0.1% Trifluoroacetic acid (TFA), 5% acetonitrile, and injected on a BEH300 C18 Acquity HPLC column (Waters) pre-equilibrated in 0.1% TFA, 34% acetonitrile. Elution was then performed with a 34 to 44% acetonitrile, 1.5-mL linear gradient in 0.1% TFA, and the proteins were detected by following the absorbance at 214 nm. The temperature of the column was maintained at 75° C., and all experiments were performed with a flow rate of 100 μL/min. (D) The quantification of the K137M microplasmin species at t=10 min by HPLC and the subsequent decrease in residual activity were combined to calculate the molar concentration of intact, active microplasmin present in the sample at each time point. The data were fitted with Equation 1 (see Example 3) to calculate the second order rate constant for autolysis (k). The open circles (○) represent the data for the K137M variant. For comparative purposes, a similar set of data obtained with another variant (K147A-R158A) is also represented (●).

FIG. **9**. Determination of the kinetic parameters for the K137M microplasmin variant. Determination of k_{cat} and K_m from the measurement of initial rates of hydrolysis (v_i) at different substrate (S-2403) concentrations. The data were fitted with Equation 2 (see Example 4).

FIG. **10**. Amino acid sequence alignment of mammalian plasminogen proteins retrieved from GenBank. The sequence alignment was run with the COBALT software (Constraint-based Multiple Alignment Tool; Papadopoulos & Agarwala, Bioinformatics 23:1073-79, 2007) available through the National Center for Biotechnology Information (NCBI) website with default settings. ▼: indication of start of Glu-plasminogen. The amino acid numbering is relative to human plasminogen.

DETAILED DESCRIPTION OF THE INVENTION

The current invention is based on the results of studying the mechanisms underlying the unforced auto-inactivation of the proteolytic activity of plasmin at neutral pH, a study for which the inventor chose to focus on microplasmin which consists mainly of the catalytic domain of plasmin. Peptide bonds susceptible to cleavage by plasmin are located at the C-terminus of lysine or arginine (Weinstein & Doolittle, 1972, Biochim Biophys Acta 258, 577-590). Nearly 10% (22 out of 230) of the amino acids of the plasmin catalytic domain (starting at amino acid position 562, a valine, in human Glu-plasminogen) are lysines or arginines. Theoretically all peptide bonds C-terminal of these lysines and arginines in one plasmin molecule can be proteolytically cleaved by another plasmin molecule.

One aspect of the invention thus relates to plasmin molecules and to plasminogen molecules, in particular plasminogen molecules that are activatable/can potentially be activated to plasmin, comprising in their catalytic domain one or more mutations of amino acids such that peptide bonds vulnerable to autoproteolytic degradation in wild-type plasmin or plasminogen are less or not vulnerable to autoproteolytic degradation in the plasmin and plasminogen molecules subject of the invention.

The invention in other words relates to an isolated plasminogen variant or plasmin obtained from it, or an isolated plasmin variant, or a proteolytically active or reversible inactive derivative of any of said plasmins, characterized in that said plasminogen variant or plasmin variant or derivative thereof is comprising in its catalytic domain the mutation of at least one internal amino acid at position P of which the peptide bond with internal amino acid at position P+1 is prone to (or sensitive to, susceptible to, or vulnerable to) autoproteolysis into an amino acid of which the peptide bond with internal amino acid at position P+1 is less or not prone (or less or not sensitive, susceptible, or vulnerable) to autoproteolysis. In particular, said internal amino acid at position P is a lysine or arginine. As reference used herein (unless stated otherwise), the catalytic domain of plasmin will be numbered relative to human plasmin, which is starting with the valine at position P=1 which is the same as the valine at position 562 of human Glu-plasminogen (see FIG. **1**). Reference can also be made herein to two different amino acid positions in the plasmin catalytic domain, which are then termed P and P', respectively.

Alternatively, the plasminogen variant, plasmin variant, or plasmin derivative according to the invention may comprise in its catalytic domain the mutation of at least two internal amino acids at position P and P' of which the peptide bond with internal amino acids at positions P+1 and P'+1 are prone to autoproteolysis into amino acids of which the peptide bond with internal amino acids at position P+1 and P'+1 is less or not prone to autoproteolysis.

After having identified the amino acids at positions P, the person skilled in the art will be able to decide easily into which other amino acid the wild-type amino acid at position P can be mutated. Such decision may, but must not necessarily imply criteria such as amino acid size, amino acid charge, amino acid polarity, and/or amino acid hydropathy index (see Table 1). In particular for plasmin and plasminogen said internal amino acid at position P in all likelihood will be a lysine or arginine, implying that these should be mutated into an amino acid different from arginine or lysine, respectively. Moreover, the availability of the crystal structure of plasminogen and the plasmin catalytic domain (MMDB ID: 12717; PDB ID: 1DDJ; Wang et al., 2001, J Mol Biol 295, 903-914) is of great value in helping identifying the mutant amino acids such that the resulting mutant plasmin or plasminogen molecule retains proteolytic activity. Furthermore, it can be expected that mutation of a wild-type amino acid at said position P into either one of the amino acids of a given group will yield similar results. Based on Table 1, said given groups can be defined as follows:

hydrophobic aliphatic amino acids: Met, Ile, Leu and Val
hydrophobic aromatic amino acids: Phe
hydrophilic acidic amino acids: Asp, Glu, Asn and Gln
hydrophilic basic amino acids: Arg, Lys and H is
moderately hydrophobic aliphatic amino acids: Gly, Ala, Ser, Thr, Cys, Pro
moderately hydrophobic aromatic amino acids: Tyr and Trp.

Of these, and for the purpose of mutation, Cys and Pro may be less favorable substitute amino acids of wild-type plasmin or plasminogen amino acids due to the creation of possible free thiol-group by a Cys, or due to more extensive disturbance of the protein structure by a Pro. Other amino acid substitutions include the mutation of a wild-type amino acid at said position P of a plasmin(ogen) catalytic domain into a non-natural or noncanonical amino acid, or into amino acid analogs, such as norleucine, norvaline, ornithine or citrulline (for more extensive list see, e.g., Hendrickson et al. 2004, Annu Rev Biochem 73, 147-176).

TABLE 1

Characteristics of amino acids.

Amino Acid			Side chain polarity	Side chain charge (at pH 7)	Hydropathy index
Alanine	Ala	A	nonpolar	neutral	1.8
Arginine	Arg	R	polar	positive	−4.5
Asparagine	Asn	N	polar	neutral	−3.5
Aspartic acid	Asp	D	polar	negative	−3.5
Cysteine	Cys	C	nonpolar	neutral	2.5
Glutamic acid	Glu	E	polar	negative	−3.5
Glutamine	Gln	Q	polar	neutral	−3.5
Glycine	Gly	G	nonpolar	neutral	−0.4
Histidine	His	H	polar	positive	−3.2
Isoleucine	Ile	I	nonpolar	neutral	4.5
Leucine	Leu	L	nonpolar	neutral	3.8
Lysine	Lys	K	polar	positive	−3.9
Methionine	Met	M	nonpolar	neutral	1.9
Phenylalanine	Phe	F	nonpolar	neutral	2.8
Proline	Pro	P	nonpolar	neutral	−1.6
Serine	Ser	S	polar	neutral	−0.8
Threonine	Thr	T	polar	neutral	−0.7
Tryptophan	Trp	W	nonpolar	neutral	−0.9
Tyrosine	Tyr	Y	polar	neutral	−1.3
Valine	Val	V	nonpolar	neutral	4.2

The inventor observed that, under the test conditions, only a limited number of autoproteolytic cleavages occur within the plasmin catalytic domain. As described in the Examples section, the current invention identified 3 hot spots of autoproteolysis. This, however, does not exclude the possibility for the existence of other peptide bonds that are autoproteolytically scissile.

Thus, in the above, said at least one internal amino acid at position P, or said at least two internal amino acids at positions P and P', are more particularly at least one or at least two chosen from:

(i) lysine at position 137 of the human plasmin catalytic domain, or the corresponding lysine or arginine of a non-human plasmin;
(ii) lysine at position 147 of the human plasmin catalytic domain, or the corresponding lysine or arginine of a non-human plasmin; or
(iii) arginine at position 158 of the human plasmin catalytic domain, or the corresponding lysine or arginine of a non-human plasmin;

wherein said human plasmin catalytic domain is starting with the amino acid valine at position 1 which is the same valine amino acid occurring at position 562 of human Glu-plasminogen. To clarify the amino acid numbering in human plasminogen and the human plasmin catalytic domain, reference is made to FIG. **1** herein.

The identification of an amino acid in a non-human plasmin(ogen) sequence which "corresponds to" (i.e. the identification of a "corresponding" amino acid) an amino acid in the human plasmin(ogen) first implies the alignment of both amino acid sequences. Such alignment may require some optimization, such as introduction of minor gaps in one or both of the aligned sequences, to result in the highest identity and homology. Secondly, the amino acid in the non-human plasmin(ogen) aligning with the amino acid in the human plasmin(ogen) is identified and is herein referred to as the "corresponding" amino acid. FIG. **10** herein depicts such an alignment of publicly available mammalian plasminogen protein sequences, and highlights the amino acids of particular interest to the current invention in the human plasminogen sequence (line 1) together with the corresponding amino acids in the non-human plasminogen sequences (lines 2-18). The amino acids of particular interest are Lys at position 698 (position 137 in the catalytic domain, see FIG. **1**), Lys at position 708 (position 147 in the catalytic domain, see FIG. **1**) and Arg at position 719 (position 158 in the catalytic domain, see FIG. **1**).

Said plasminogen variant, plasmin variant, or plasmin derivative according to the invention may be one wherein said at least one internal amino acid at position P is the lysine at position 147 of the human plasmin catalytic domain, or is the corresponding lysine or arginine of a non-human plasmin catalytic domain. It may optionally comprise further a mutation of the internal amino acids at positions 137 and/or 158 of the human catalytic domain or of the corresponding lysines and/or arginines of a non-human plasmin catalytic domain. Herein said human plasmin catalytic domain is starting with the amino acid valine at position 1 which is the same valine amino acid occurring at position 562 of human Glu-plasminogen.

Said plasminogen variant, plasmin variant, or plasmin derivative according to the invention may alternatively be one wherein:

(i) if the mutation of said at least one internal amino acid at position P is the mutation of the lysine at position 137 of the human plasmin catalytic domain (which is amino acid position 698 relative to human Glu-plasminogen) into an amino acid rendering the peptide bond between amino acids 137 and 138 resistant or more resistant to autoproteolysis, said plasminogen variant, plasmin variant or plasmin derivative comprises an intact activation site at amino acid positions 561 and 562 (relative to human Glu-plasminogen), and, when amino acids at position 536 and 541 (relative to human Glu-plasminogen) outside the catalytic domain are present, said amino acids are the wild-type cysteines, or

(ii) if the mutation of said at least one internal amino acid at position P is the mutation of the arginine at position 158 of the human plasmin catalytic domain (which is amino acid position 719 relative to human Glu-plasminogen) into an alanine or glutamate, then at least one other internal amino acid of the human plasmin catalytic domain at a position P' of which the peptide bond with internal amino acid at position P'+1 is prone to autoproteolysis is mutated into an amino acid of which the peptide bond with internal amino acid at position P'+1 is less or not prone to autoproteolysis.

The variants described in (i) and (ii) above may optionally further comprise a mutation of the internal amino acid at position 147 of the human catalytic domain or of the corresponding lysine or arginine of a non-human plasmin catalytic domain, wherein said human plasmin catalytic domain is starting with the amino acid valine at position 1 which is the same valine amino acid occurring at position 562 of human Glu-plasminogen.

In any of the above-described plasminogen variants, plasmin variants, or plasmin derivatives said lysine at position 137 of the human catalytic domain, or of the corresponding lysine or arginine of a non-human plasmin catalytic domain, may be mutated into an amino acid of the groups of hydrophobic aliphatic amino acids, hydrophobic aromatic amino acids, hydrophilic acidic amino acids, hydrophilic basic amino acids other than lysine, moderately hydrophobic aromatic amino acids, and moderately hydrophobic aliphatic amino acids. In particular, said lysine may e.g. be mutated into an amino acid chosen from Ala, Glu, Phe, H is, Ile, Met, Gln or Arg.

In any of the above-described plasminogen variants, plasmin variants, or plasmin derivatives said lysine at position 147 of the human catalytic domain, or of the corresponding lysine or arginine of a non-human plasmin catalytic domain, may be mutated into an amino acid of the groups of hydrophobic aliphatic amino acids, hydrophobic aromatic amino acids, hydrophilic acidic amino acids, hydrophilic basic amino acids other than lysine, moderately hydrophobic aromatic amino acids, and moderately hydrophobic aliphatic amino acids. In particular, said lysine may e.g. be mutated into an amino acid chosen from Ala, Glu, Gln, H is, Ile or Phe.

In any of the above-described plasminogen variants, plasmin variants, or plasmin derivatives said arginine at position 158 of the human catalytic domain, or of the corresponding lysine or arginine of a non-human plasmin catalytic domain, may be mutated into an amino acid of the groups of hydrophobic aliphatic amino acids, hydrophobic aromatic amino acids, hydrophilic acidic amino acids, hydrophilic basic amino acids, moderately hydrophobic aromatic amino acids, and moderately hydrophobic aliphatic amino acids. In particular, said arginine may e.g. be mutated into an amino acid chosen from Ala, Glu, Gln, Ile, Phe or His.

"Plasmin", also known as fibrinolysin or lysofibrin, is a serine-type protease which results from the activation of the zymogen plasminogen. Activation is the result of a proteolytic cleavage between amino acids 561 and 562 (numbering relative to human Glu-plasminogen). Plasmin carries a heavy chain comprising 5 kringle domains and a light chain comprising the catalytic domain. Plasminogen can be enriched from blood plasma, e.g., via lysine affinity-chromatography (Deutsch & Mertz, 1970, Science 170, 1095-1096). Truncation of the plasmin molecule (outside and/or inside the plasmin catalytic domain) is possible as long as the catalytic domain remains functional, such truncation thus results in the formation of a "proteolytically active derivative" of plasmin. As such, one or more of the 5 kringle domains can be deleted wholly or partially. Truncated plasmins lacking one or more kringle domains and/or lacking parts of one or more kringle domains therefore are envisaged by the current invention as examples of proteolytically active derivatives of plasmin Examples of truncated variants of plasmin include, but are not limited to, "midiplasmin", "miniplasmin", "microplasmin", and "delta-plasmin". Midiplasmin is basically lacking kringle domains 1 to 3 (e.g. Christensen et al., 1995, Biochem J 305, 97-102). Miniplasmin was originally obtained by limited digestion of plasmin with elastase and is basically lacking kringle domains 1 to 4 (e.g. Christensen et al., 1979, Biochim Biophys Acta 567, 472-481; Powell & Castellino, 1980, J Biol Chem 255, 5329). Miniplasmin has subsequently been produced recombinantly (WO 2002/050290). Microplasmin was originally obtained by incubation of plasmin at elevated pH and is basically lacking all kringle domains (e.g. WO 89/01336). Whereas the microplasmin obtained from incubation of plasmin at elevated pH is containing the 30-31 carboxy-terminal amino acids of the heavy chain, a recombinantly produced microplasmin variant is containing the 19 carboxy-terminal amino acids of the heavy chain (WO 2002/050290). Delta-plasmin is a recombinant version of plasmin in which kringle domain 1 is linked directly with the catalytic domain (WO 2005/105990). The above described truncated variants of plasmin are obtained by activation of "midiplasminogen", "miniplasminogen", "microplasminogen" and "delta-plasminogen", respectively. In order to be activatable, a truncated plasminogen needs to comprise a minimum number of amino acids of the linker between the kringle 5 domain and the catalytic domain (see, e.g., Wang et al., 1995, Protein Science 4, 1758-1767). In the context of the present invention it may be desired that the plasminogen comprises an "intact activation site", which implies that at least amino acids 561 and 562 (relative to human Glu-plasminogen; or the corresponding amino acids in non-human plasminogen) are such that activation/conversion of plasminogen to plasmin can occur, albeit possibly with different kinetics, as it occurs in wild-type plasmin. As alternative to plasmin or an active truncated variant thereof, an activatable plasminogen or a truncated variant thereof can be used in the context of the current invention (see, e.g. EP 0480906; U.S. Pat. No. 5,304,383; EP 0631786; U.S. Pat. No. 5,520,912; U.S. Pat. No. 5,597,800; U.S. Pat. No. 5,776,452). "Plasminogen" refers to any form of plasminogen e.g. Glu-plasminogen or Lys-plasminogen (starting with Arg at position 68 or Lys at positions 77 or 78). When using activatable plasminogen or an activatable truncated variant thereof, the activation to plasmin may be delayed and will typically occur after contacting it with an organ, tissue or body fluid, i.e. after administration to a subject. In yet another alternative, the plasmin or an active truncated variant thereof can be substituted in the context of the current invention for an activatable plasminogen or an activatable truncated variant thereof in conjunction with a plasminogen activator (such as tissue plasminogen activator (tPA), urokinase, streptokinase or staphylokinase, or any variant thereof; see, e.g. U.S. Pat. No. 6,733,750; U.S. Pat. No. 6,585,972; U.S. Pat. No. 6,899,877; WO 03/33019). In yet a further alternative, a mixture of any of (i) plasmin or derivative thereof, (ii) activatable plasminogen or an activatable derivative thereof, and, optionally (iii) a plasminogen activator can be used in the context of the current invention (see, e.g. US 2004/0081643). In order to ensure stability of the plasmin (or plasminogen), it will generally be stored at lowered temperatures (e.g. +4 degrees Celsius or −20 degrees Celsius). The storage composition may be a stabilizing composition such as a low pH composition (pH 4 or lower; obtained by e.g. 1 mM to 250 mM of an acid such as citric acid, see, e.g. Castellino & Sodetz, 1976, Methods Enzymol 45, 273-286; WO 01/36608; WO 01/36609; WO 01/36611) or a high glycerol content composition (30-50% v/v, e.g., Castellino & Sodetz, 1976, Methods Enzymol 45, 273-286), alternatively in or in conjunction with one or more further stabilizer compositions comprising e.g. an amino acid (e.g. lysine or an analogue thereof such as EACA or tranexamic acid), a sugar (e.g. mannitol) or any stabilizer as known in the art (e.g. dipeptides, WO 97/01631). Further included in the genus "plasmin" is any active derivative thereof (or of an active truncated plasmin variant), or similar derivative of activatable plasminogen (or of activatable truncated variant thereof). Such derivates include e.g. labeled plasmin or plasminogen (or truncated variants thereof) such as Tc^{99}-labeled plasmin (Deacon et al., 1980, Br J Radiol 53, 673-677) or pegylated or acylated plasmin or plasminogen (or truncated variants thereof; EP 9879, WO 93/15189). Any other label (radioactive, fluorescent, etc.) may also be used to produce a plasmin or plasminogen derivative. Said derivatives further include hybrid or chimeric plasmin or plasminogen molecules comprising e.g. a truncated plasmin or plasminogen according to the invention fused with e.g. a fibrin-binding molecule (such as kringle 2 of tPA, an apolipoprotein kringle, the finger domain of tPA or fibronectin or the Fab domain of a fibrin-binding antibody).

Comparison of the autoproteolytic resistance (i.e. stability) of wild-type plasmin and of plasmin variants or plasmin derivatives according to the invention can be performed in a similar way as as for comparing proteolytic activity, e.g., in a chromogenic activity assay or a biological substrate assay based on e.g. fibrin, fibrinogen or fibronectin.

In order to determine autoproteolytic resistance, the autolysis rate constant can be determined. It is envisaged that the plasmin variants according to the invention, including the plasmins obtained from the plasminogen variants according to the invention, or any of the plasmin derivatives according to the invention may be characterized by an autolysis rate constant that is at least 5%, or at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 75%, 80%, 90%, 95%, 99% or 99.5% lower than the autolysis rate constant of wild-type plasmin, or, alternatively, by an autolysis rate constant that is at most 95%, or at most 0.5%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 75%, 80%, or 90% of the autolysis rate constant of wild-type plasmin. In order to determine the indicated percentage, the calculation can be done based on the absolute autolysis rate constant numbers. For example, wild-type microplasmin has an autolysis rate constant of 230 $M^{-1}s^{-1}$, whereas the microplasmin variant K137M has an autolysis rate constant of 1 $M^{-1}s^{-1}$ (see Example 3/Table 3). The autolysis rate constant of the K137M variant therefore is 0.43% of the autolysis rate constant of wild-type microplasmin.

Further, any of the plasmin variants according to the invention, including the plasmins obtained from the plasminogen variants according to the invention, or derivatives of any of said plasmins may retain proteolytic activity different (higher or lower) from the proteolytic activity of wild-type plasmin, such as determined with e.g. a chromogenic activity assay or a biological substrate assay based on e.g. fibrin, fibrinogen, fibronectin, gelatin, laminin or collagen.

The proteolytic activities of the plasmin variants according to the invention, including the plasmins obtained from the plasminogen variants according to the invention, or any of the plasmin derivatives according to the invention may be compared to the proteolytic activity of wild-type plasmin by means of the catalytic constant k_{cat} which is a measure of the number of substrate molecule each enzyme site converts to product per unit time. Thus, any of the plasmin variants according to the invention, including the plasmins obtained from the plasminogen variants according to the invention, or any of the plasmin derivatives according to the invention may be characterized by a k_{cat} value which is in the range of +100% to −90%, or +50% to −50% of the k_{cat} value of wild-type plasmin, i.e., characterized by a k_{cat} value in the range of 10% to 200%, or 50% to 150% of the k_{cat} value of wild-type plasmin. In order to determine the indicated percentage, the calculation is done on the absolute k_{cat} numbers. For example, wild-type microplasmin has a k_{cat} of 46 s^{-1}, whereas the microplasmin variant K137M has a k_{cat} of 36s^{-1} (see Example 4/Table 3). The k_{cat} of the K137M variant therefore is 78.3% of the k_{cat} of wild-type microplasmin.

Another way of comparing proteolytic activity of the plasmin variants according to the invention, including the plasmins obtained from the plasminogen variants according to the invention, or any of the plasmin derivatives according to the invention to proteolytic activity of wild-type plasmin includes comparing k_{cat}/K_m (Table 3). An up to 1000-times or up to 500-times lower k_{cat}/K_m of a plasmin variants according to the invention, including the plasmins obtained from the plasminogen variants according to the invention, or any of the plasmin derivatives according to the invention compared to the k_{cat}/K_m of wild-type plasmin can still be acceptable (see further).

Further, any of the plasmin variants according to the invention, including the plasmins obtained from the plasminogen variants according to the invention, or any of the plasmin derivatives according to the invention may be characterized by the combination of the above-defined autolysis rate constant and catalytic constant k_{cat}.

Alternatively, any of the plasmin variants according to the invention, including the plasmins obtained from the plasminogen variants according to the invention, or any of the plasmin derivatives according to the invention may be compared to wild-type plasmin by combining autolytic rate constant data and k_{cat}/K_m data. For example, a plasmin variant with a 20-times lower autolytic rate constant compared to wild-type plasmin, and with a 10-times lower k_{cat}/K_m compared to wild-type plasmin will be 2-times better than the wild-type plasmin. Obviously depending on the ultimate use, a very stable plasmin (i.e. no or nearly no autoproteolytic degradation) with low proteolytic activity may be highly desired, e.g., in cases where low but prolonged plasmin activity is desired or even required to achieve the intended clinical effect. Such highly stable plasmin variants with low proteolytic activity would as such virtually equal slow-release formulations without the real need to actually use a slow-release carrier or adjuvant.

Yet another alternative to compare any of the plasmin variants according to the invention, including the plasmins obtained from the plasminogen variants according to the invention, or any of the plasmin derivatives according to the invention may be compared to wild-type plasmin by combining autolytic rate constant data and k_{cat} data.

Obviously, for any comparative measurements such as described above it is desirable to compare plasmin variants with their closest wild-type plasmin, e.g., to compare a microplasmin variant with wild-type microplasmin, or a miniplasmin variant with wild-type miniplasmin. Furthermore obvious, for any activity measurement, a reversibly inactivated derivative of a plasmin variant according to the invention should first be activated by removing the cause of reversible inactivation (e.g. acylation or non-optimal pH).

Any of the plasminogen variants according to the invention or plasmins obtained therefrom, of the plasmin variants according to the invention may be Glu-plasminogen of Glu-plasmin, Lys-plasminogen or Lys-plasmin, midiplasminogen or midiplasmin, miniplasminogen or miniplasmin, microplasminogen or microplasmin, deltaplasminogen or deltaplasmin.

Many assays exist to determine whether or not a plasmin species is proteolytically active. Easy and straightforward assays are based on the digestion of a chromogenic substrate by plasmin present in a sample; chromogenic substrates include S-2403 (Glu-Phe-Lys-pNA) and S-2251 (Val-Leu-Lys-pNA) which release p-nitroaniline (pNA) upon proteolytic cleavage. The amount of pNA formed can be measured by light absorbance at 405 nm. An alternative assay for determining plasmin activity is a potentiometric assay. Colorimetric (using a chromogenic substrate) and potentiometric assays are described in e.g., Castellino & Sodetz (1976, Methods Enzymol 45, 273-286). A further alternative assay for determining plasmin activity is a caseinolytic assay (e.g., Robbins & Summaria, 1970, Methods Enzymol 19, 184-199; Ruyssen & Lauwers, 1978, Chapter IX—Plasmin, In "Pharmaceutical Enzymes", Story-Scientia, Gent, Belgium, pp. 123-131). Yet another alternative assay for determining plasmin activity is a fibrinolytic assay (e.g., Astrup & Mullertz, 1952, Arch Biochem Biophys 40, 346-351). Further activity assays could be easily designed using other protein substrates. Clearly, such assays may also be used to follow disappearance of plasmin proteolytic activity over time due to autoproteolytic degradation of the enzyme. As an alternative for assessing stability of a plasmin variant or any active truncated variant or derivative thereof of the current invention, said plasmin variant may be incubated in the presence of wild-type plasmin and the resistance of the plasmin variant to digestion by wild-type plasmin can be monitored.

The use of plasmin in the removal of necrotic elements or debris from lesions, wounds, ulcerating wounds (such as ulcerating stitched wounds) etc. has been described in e.g. U.S. Pat. No. 3,208,908. Similarly, topical application of plasmin-comprising therapeutic preparations for the treatment of burns was disclosed in e.g. U.S. Pat. No. 4,122,158. Debridement refers to the removal of dead, damaged and/or infected tissue in order to improve or increase the healing of remaining healthy tissue. Such removal may be obtained by surgical, mechanical or chemical means, or by means of certain species of live maggots that selectively eat necrotic tissue (maggot therapy). Debridement may also be performed using enzymes or may be assisted by enzymes, a process referred to as enzymatic debridement. Debridement is an important aspect in the healing process of burns and other serious wounds and it is used as well in the treatment of some types of snake bites. The application of plasmin (or of any variant or derivative thereof or alternative therefore as described above) in enzymatic debridement (alone or in combination with other types of debridement) is particularly useful in promoting or facilitating wound healing and as an adjunct in surgical procedures such as skin grafting.

A more commonly known use of plasmin (or of any variant or derivative thereof or alternative therefore as described above) relates in general terms to the treatment of (a) pathological deposit(s) of fibrin. Fibrin deposits can result from a wide variety of pathological situations in the body. For example, fibrin-containing blood clots can form in vessels in tissue resulting in deep vein, coronary artery, cerebral artery or retinal vein occlusion or thrombosis. Small accumulations of fibrin precede, and may provide, warning of impending catastrophic thrombosis. Examples include unstable angina pectoris, which is considered a warning of impending coronary thrombosis and transient ischemic attacks, which may precede strokes. Fibrin is furthermore frequently deposited in tissue in association with inflammation associated with many disease processes including infection, autoimmune disease and cancer. Another situation where fibrin is deposited is around abscesses caused by infection with microorganisms. Fibrin deposits are furthermore frequently found associated with certain solid tumors. Fibrin deposition may also occur during the healing of any type of wound. Yet another situation of fibrin deposition is the accumulation of fibrin in a retinal vein, which can lead to retinal degeneration, disturbed vision or even loss of vision. The term pathological fibrin deposit further encompasses such deposits as formed or as present in or at the tip of a catheter, catheter device or other implant such as prosthetic vessels and grafts of synthetic, human or animal origin and effectively blocked by an occlusion comprising fibrin. The term "catheter device" refers to any catheter or tube-like device that may enter the body, including arterial catheters, cardiac catheters, central venous catheters, intravenous catheters, peripherally inserted central catheters, pulmonary artery catheters, tunneled central venous catheters and arterio-venous shunts.

Among the various factors encouraging the process of thrombosis, i.e. the formation of a thrombus or hemostatic plug, are: (1) damage to the endothelial cell lining of the affected blood vessel, (2) an increase in the clotting properties of the blood, and (3) stagnation of blood in the affected blood vessel. Thrombosis can start as a very small lump attached to the damaged part of the blood vessel lining. Its presence encourages further thrombosis to occur, and has the effect of causing a slow-down of blood flow by reducing the inner diameter of the vessel. Further growth of the initially small thrombus often leads to total or almost total blockage of the affected blood vessel. If thrombosis takes place in one of the arteries, the tissues supplied by that artery may be deprived of oxygen and nutrition, causing damage or death of the tissue (gangrene). The severity of the damage depends upon the position and size of the thrombosis, the speed at which it grows and whether the affected area has only one artery or is supplied by collateral blood vessels. If the vessel to a vital organ is affected, e.g. the heart or the brain, the person may be severely crippled or die. Sometimes a thrombus may contain infective organisms such as bacteria, and septic thrombosis may occur, with the formation of pus and infection of the surrounding tissues.

Further uses of plasmin (or of any variant or derivative thereof or alternative therefore as described above) include the reduction of the level of circulating fibrinogen (e.g. WO 93/07893) and its use as an α2-antiplasmin inhibitor (reported to reduce the size of cerebral infarct after ischemic stroke; WO 00/18436).

Yet another use of plasmin (or of any variant or derivative thereof or alternative therefore as described above) is related to the induction of posterior vitreous detachment (PVD) and/or vitreous liquefaction in the eye as an alternative for or as adjunct to mechanical vitrectomy (WO 2004/052228; U.S. Pat. No. 6,733,750; U.S. Pat. No. 6,585,972; U.S. Pat. No. 6,899,877; WO 03/33019; WO 2006/122249; WO 2007/047874; U.S. Pat. No. 5,304,118; US 2006/0024349; US 2003/0147877). Vitrectomy and/or vitreous liquefaction is of benefit for a number of eye conditions such as vitreous floaters (motile debris/deposits of vitreous within the normally transparent vitreous humour which can impair vision), retinal detachment (a blinding condition which may be caused by vitreal traction), macular pucker (scar tissue on macula; macula is required for sharp, central vision; macular pucker is also known as epi- or preretinal membrane, cellophane maculopathy, retina wrinkle, surface wrinkling retinopathy, premacular fibrosis, or internal limiting membrane disease), diabetic retinopathy (proliferative or non-proliferative) which may result in vitreal hemorrhage and/or formation of fibrous scar tissue on the retina (which may cause retinal detachment), macular holes (hole in macula causing a blind spot and caused by vitreal traction, injury or a traumatic event), vitreous hemorrhage (caused by diabetic retinopathy, injuries, retinal detachment or retinal tears, subarachnoidal bleedings (Terson syndrome), or blocked vessels), subhyaloid hemorrhage (bleeding under the hyaloid membrane enveloping the vitreous), macular edema (deposition of fluid and protein on or under the macula of the eye) and macular degeneration (starting with the formation of drusen; occurs in dry and wet form; if correlated with age coined age-related macular degeneration). Other eye-applications of plasmin include the maintenance or rescue of a filtering bleb after trabeculectomy surgery (performed to reduce intra-ocular pressure), see e.g. WO 2009/073457.

Another further use of plasmin (or of any variant or derivative thereof or alternative therefore as described above) resides in diagnosis, more particularly appropriately labeled (e.g. Tc^{99}-labeled, see above) plasmin (or any variant or derivative thereof or alternative therefore as described above) may be applied for detecting pathological fibrin deposits. When applying a truncated plasmin or plasminogen variant according to the current invention in such diagnosis, care should be taken that said variant still comprises a fibrin-binding site (whether or not from plasmin itself or added to e.g. the plasmin catalytic domain by creating a hybrid molecule).

The plasmin or any variant or derivative thereof or alternative therefore according to the invention may be stored in a pharmaceutically acceptable carrier, diluent or adjuvant. Such carrier, diluent or adjuvant may consist of or comprise an acidic low buffer such as 1-100 mM acetate or citrate. When acidic, the pharmaceutically acceptable carrier, diluent or adjuvant may have a pH of 2.5 to 4.0, such as at a pH of 3.0 to 3.5, or such as a pH of 3.1. Useful acidic compounds include acetic acid, citric acid, hydrochloric acid, lactic acid, malic acid, tartaric acid or benzoic acid. Formic acid may be used but care should be taken that this compound is not inducing proteolytic cleavage at the C-terminus of Asp-residues. The pharmaceutically acceptable carrier, diluent or adjuvant, acidic or neutral, may comprise one or more amino acids such as serine, threonine, methionine, glutamine, glycine, isoleucine, valine, alanine, aspartic acid, lysine, histidine or any derivatives or analogues thereof. The pharmaceutically acceptable carrier, diluent or adjuvant may comprise a carbohydrate such as a monosaccharide, disaccharide, polysaccharide or polyhydric alcohol. Examples include sugars such as sucrose, glucose, fructose, lactose, trehalose, maltose and mannose, sugar alcohols such as sorbitol and mannitol and polysaccharides such as dextrins, dextrans, glycogen, starches and celluloses. The pharmaceutically acceptable carrier, diluent or adjuvant may comprise compounds such as glycerol, niacinamide, glucosamine, thiamine, citrulline, inorganic salts (such as sodium chloride, potassium chloride, magnesium chloride, calcium chloride), benzyl alcohol or benzoic acid. The pharmaceutically acceptable carrier, diluents or adjuvant may comprise compounds such as ϵ-aminocaproic acid (EACA) and/or tranexamic acid (see also above & Background section). Some of these compounds may be used as stabilizer of a plasmin or any variant or derivative thereof or alternative therefore as described above.

In view of the above, another aspect of the invention relates to the isolated plasminogen, plasmin, or any variant or derivative thereof or alternative therefore according to the invention, or a combination of any thereof for use as a medicament.

A further aspect of the invention relates to compositions comprising the isolated plasminogen, plasmin, or any variant or derivative thereof or alternative therefore according to the invention, or a combination of any thereof, and at least one of a pharmaceutically acceptable diluent, carrier or adjuvant. In a further embodiment, said composition may additionally comprise at least one of an anticoagulant, a further thrombolytic agent, an anti-inflammatory agent, an antiviral agent, an antibacterial agent, an antifungal agent, an anti-angiogenic agent, an anti-mitotic agent, an antihistamine or an anaesthetic.

In an embodiment to the above-described two aspects of the invention, the isolated plasminogen, plasmin, or any variant or derivative thereof or alternative therefore according to the invention, or of a combination of any thereof, or the composition according to the invention may be used in any clinically relevant setting such as for treating a pathological fibrin deposit, for inducing posterior vitreous detachment in the eye, for inducing liquefaction of the vitreous in the eye, as adjunct to and facilitating vitrectomy in the eye, for inducing posterior vitreous detachment, for resolving vitreomacular adhesion, for closing macular holes, for enzymatic debridement, for reducing circulating fibrinogen, for reducing α2-antiplasmin levels, or in conjunction with trabeculectomy.

In another embodiment to the above-described two aspects of the invention, the isolated plasminogen, plasmin, or any variant or derivative thereof or alternative therefore according to the invention, or of a combination of any thereof, or the composition according to the invention may be used for prophylactic purposes or in methods for prophylactic treatment. Prophylactic uses include reducing the risk of development of a pathological fibrin deposit in a mammal having an increased risk of developing it (such as an obese mammal, a mammal not doing sufficient physical exercise or a mammal scheduled to undergo a major surgical event or operation). Other prophylactic uses include the induction of posterior vitreous detachment and/or vitreous liquefaction in an apparent healthy eye of a mammal of which the companion eye is/was diagnosed to require induction of posterior vitreous detachment and/or vitreous liquefaction.

Alternatively, the invention relates to methods for treating, dissolving, loosening, macerating, lysing, inducing or promoting lysis of a pathological fibrin deposit in a subject, said methods comprising contacting said fibrin deposit with an effective amount of the isolated plasminogen, plasmin, or any variant or derivative thereof or alternative therefore according to the invention, or of a combination of any thereof, said contacting resulting in the treatment, dissolution, loosening, maceration, lysis, or induction or promotion of lysis of said pathological fibrin deposit.

The invention further relates to methods for inducing posterior vitreous detachment in the eye and/or for inducing liquefaction of the vitreous in the eye, or for facilitating surgical vitrectomy in the eye in a subject, said methods comprising contacting an eye of said subject in need of such treatment with an effective amount of the isolated plasminogen, plasmin, or any variant or derivative thereof or alternative therefore according to the invention or of a combination of any thereof, said contacting resulting in the induction of said posterior vitreous detachment and/or of said liquefaction of the vitreous, or in the facilitation of said surgical vitrectomy.

The invention also relates to methods for enzymatic debridement of injured tissue of a subject, said method comprising contacting said injured tissue with an effective amount of the isolated plasminogen, plasmin, or any variant or derivative thereof or alternative therefore according to the invention, or of a combination of any thereof, said contacting resulting in said enzymatic debridement of said injured tissue.

Other methods of the invention are treating or preventing any other clinically relevant indication, including methods for reducing circulating fibrinogen, or for reducing α2-antiplasmin levels in a subject, said methods comprising contacting a subject in need of such treatment with an effective amount of the isolated plasminogen, plasmin, or any variant or derivative thereof or alternative therefore according to the invention, or of a combination of any thereof, said contacting resulting in said reduction of circulating fibrinogen or of said α2-antiplasmin levels.

In general, the medicament or composition of the invention comprising a plasmin (or any variant or derivative thereof or alternative therefore) according to the invention may, depending on its ultimate use and mode of administration, comprise one or more further active ingredients such as an anticoagulant, a further thrombolytic agent, an anti-inflammatory agent, an antiviral agent, an antibacterial agent, an antifungal agent, an anti-angiogenic agent, an anti-mitotic agent, an antihistamine or anesthetic.

"Anticoagulants" include hirudins, heparins, coumarins, low-molecular weight heparin, thrombin inhibitors, platelet inhibitors, platelet aggregation inhibitors, coagulation factor inhibitors, anti-fibrin antibodies and factor VIII-inhibitors (such as those described in WO 01/04269 and WO 2005/016455).

"Thrombolytic agents" include wild-type plasmin, wild-type plasminogen, urokinase, streptokinase, tissue-type plasminogen activator (tPA or alteplase), urokinase-type plasminogen activator (uPA) and staphylokinase or any variant or derivative of any thereof such as APSAC (anisoylated plasminogen streptokinase activator complex), reteplase, tenecteplase, scuPA (single chain uPA), or a combination of any thereof.

"Anti-inflammatory agents" include steroids (e.g. prednisolone, methylprednisolone, cortisone, hydrocortisone, prednisone, triamcinolone, dexamethasone) and non-steroidal anti-inflammatory agents (NSAIDs; e.g. acetaminophren, ibuprofen, aspirin).

"Antiviral agents" include trifluridine, vidarabine, acyclovir, valacyclovir, famciclovir, and doxuridine.

"Antibacterial agents" or antibiotics include ampicillin, penicillin, tetracycline, oxytetracycline, framycetin, gatifloxacin, gentamicin, tobramycin, bacitracin, neomycin and polymyxin.

"Anti-mycotic/fungistatic/antifungal agents" include fluconazole, amphotericin, clotrimazole, econazole, itraconazole, miconazole, 5-fluorocytosine, ketoconazole and natamycin.

"Anti-angiogenic agents" include antibodies (or fragments thereof) such as anti-VEGF (vascular endothelial growth factor) or anti-P1GF (placental growth factor) antibodies and agents such as macugen (pegaptanib sodium), trypthophanyl-tRNA synthetase (TrpRS), anecortave acetate, combrestatin A4 prodrug, AdPEDF (adenovector capable of expressing pigment epithelium-derived factor), VEGF-trap, inhibitor of VEGF receptor-2, inhibitors of VEGF, P1GF or TGF-13, Sirolimus (rapamycin) and endostatin.

"Anti-mitotic agents" include mitomycin C and 5-fluorouracyl.

"Antihistamine" includes ketitofen fumarate and pheniramine maleate.

"Anesthetics" include benzocaine, butamben, dibucaine, lidocaine, oxybuprocaine, pramoxine, proparacaine, proxymetacaine, tetracaine and amethocaine.

"Contacting", when used herein, means any mode of administration that results in interaction between a composition such as a medicament and the tissue, body fluid, organ, organism, etc. with which said composition is contacted. The interaction between the composition and the tissue, body fluid, organ, organism, etc can occur starting immediately or nearly immediately with the administration of the composition, can occur over an extended time period (starting immediately or nearly immediately with the administration of the composition), or can be delayed relative to the time of administration of the composition.

Any method of contacting a pathological fibrin deposit that provides (either immediately, delayed or over an extended time period) an effective amount of a plasmin (or any variant or derivative thereof or alternative therefore) to such fibrin deposit can be utilized. If such fibrin deposit is associated with a blood clot, the plasmin (or any variant or derivative thereof or alternative therefore) can be delivered intra-arterially, intravenously, or locally (within short distance of the clot or even in the clot) by means of injection and/or infusion and/or a catheter.

When using plasmin (or any variant or derivative thereof or alternative therefore) in enzymatic debridement, it may be included in a gel-like composition capable of being applied topically, or may be applied in liquid form.

Any method of contacting the eye vitreous and/or aqueous humor that provides (either immediately, delayed or over an extended time period) an effective amount of a plasmin (or any variant or derivative thereof or alternative therefore) to the vitreous and/or aqueous humor can be utilized. One method of contacting the vitreous and/or aqueous humor is by one or more intraocular injections directly into the vitreous and/or aqueous humor. Alternatively, said contacting may involve subconjunctival, intramuscular or intravenous injections. A further alternative contacting method involves placing an intra-vitreal implantable device such as OCUSERT® (Alza Corp., Palo Alto, Calif.) or VITRASERT® (Bausch & Lomb Inc., Rochester, N.Y.). Contacting the vitreous and/or aqueous humor with an effective amount of a plasmin (or any variant or derivative thereof or alternative therefore) may be in a continuous fashion using a depot, sustained release formulation or any implantable device suitable thereto.

The term "effective amount" refers to the dosing regimen of the medicament according to the invention, in particular of the active ingredient of the medicament according to the invention, i.e., plasmin or an active truncated variant thereof (or any alternative therefore as described above). The effective amount will generally depend on and will need adjustment to the mode of contacting or administration and the condition to be treated. The effective amount of the medicament, more particular its active ingredient, is the amount required to obtain the desired clinical outcome or therapeutic or prophylactic effect without causing significant or unnecessary toxic effects. To obtain or maintain the effective amount, the medicament may be administered as a single dose or in multiple doses. The effective amount may further vary depending on the severity of the condition that needs to be treated or the expected severity of the condition that needs to be prevented; this may depend on the overall health and physical condition of the patient and usually the treating doctor's or physician's assessment will be required to establish what is the effective amount. The effective amount may further be obtained by a combination of different types of administration. The medicament may be administered as a solution (liquid or semi-liquid, e.g., gel-like or in dispersion or suspension, colloidal, in emulsion, nanoparticle suspension) or as a solid (e.g. tablet, minitablet, hard- or soft-shelled capsules).

For purposes of thrombolysis, plasmin dosage and duration of plasmin therapy will typically depend on the size and location of the blood clot as well as on the size, weight and age of the patient. If a clot is venous, treatment with plasmin may continue for days whereas only hours of plasmin therapy may be required if the clot is arterial. A myocardial infarction may be treated with a short single dose treatment whereas conditions such as thrombophlebitis and pulmonary embolism may require longer multiple dose treatment. Prolonged continuous and/or intermittent thrombolytic plasmin therapy may be applied to treat a coronary occlusion or in case of prophylactic therapy in order to reduce the risk of clot formation in subjects known to have an increased risk to develop clot formation. A further factor influencing plasmin dosage includes the circulating levels plasmin inhibitors such as α2-antiplasmin and/or α2-macroglobulin, the initial level of which being patient-dependent. It may be advisable to adjust the plasmin dosage such that no more than 15% of the total circulating α2-antiplasmin is remaining in order to achieve efficient thrombolytic therapy. For the purpose of inducing thrombolysis, a contacting method delivering a plasmin or any variant or derivative thereof or alternative therefore at a short distance proximal to a thrombus may be advantageous as the exposure to serum inhibitors is reduced. Such contacting method typically involves delivery via a catheter device. For use in thrombolyis, typical plasmin dosages range from 500 microgram/body weight to 10 milligram/kg body weight given as a single bolus or divided over 1 initial bolus injection followed by 1 or more repeat bolus injections. Plasmin may alternatively be administered over an extended time period, e.g. by infusion or by drug delivery micropump. Plasmin dosages for continued administration may range from 1 to 10 mg/kg/hour.

A typical plasmin dosage for inducing posterior vitreous detachment, vitreous liquefaction, clearance of vitreal blood or hemorrhages, or clearance of toxic materials or foreign substances from the vitreous cavity may be in the range of about 0.1 microgram to about 250 microgram per eye per dose, which can be delivered in a diluent or carrier volume of about 50 microliter to about 300 microliter per eye per dose. The diluent or carrier may e.g. be a sterile Balanced Salt Solution (BSS or BSS Plus), a physiologic saline solution or a solution containing 1-10 mM citric acid. In one embodiment plasmin is delivered to the eye in a dose of 125 microgram contained in 0.1 mL diluent or carrier. In the case of vitrectomy, said plasmin may be delivered to the eye 15 to 300 minutes, or 15 to 120 minutes prior to the vitrectomy. When using plasminogen as an alternative source for plasmin (see "plasmin" definition), up to 250 microgram of plasminogen can be introduced per eye and said plasminogen may be accompanied by up to 2000 IU of urokinase or streptokinase as plasminogen activator or by up to 25 microgram of tPA. When used in the eye, plasmin or plasminogen administration may further be accompanied by administration of a gaseous adjuvant such as air, an expanding gas or liquefiable gas, or mixtures thereof, as long as it is non-toxic to the eye. Other suitable gaseous materials include SF6 (sulfur hexafluoride) and perfluorocarbons, such as C2F6 (hexafluoroethane), C3Fs (octafluoropropane), C4Fs (octafluorocyclobutane), oxygen, nitrogen, carbon dioxide, argon, and other inert gases. The volume of the gaseous material that is introduced into the eye can vary depending on the gaseous material, the patient, and the desired result. For example, the volume of air that is injected into the posterior chamber can range from about 0.5 mL to about 0.9 mL. Other gaseous materials, such as SF6 and perfluorocarbon gases can range from about 0.3 mL to 0.5 mL. Preferably, the gaseous material is introduced into the posterior chamber of the eye in an amount sufficient to compress the vitreous against the posterior hyaloid and form a cavity in the vitreous without damaging the eye. In preferred embodiments, the gaseous adjuvant is introduced into the vitreous to form a cavity that fills about 40% to about 60% of the internal volume of the intraocular cavity.

The above recited dosages are indicative values not meant to be limiting in any way. Said dosages furthermore refer to wild-type plasmin or plasminogen or any active or activatable truncated variant thereof. When using a plasmin with increased stability according to the invention (or any variant or derivative thereof or alternative therefore), and depending on the ultimate stability and residual activity of a plasmin according to the invention, dosages may be similar, higher or lower to obtain the same or better overall clinical effect as obtained with wild-type plasmin. Dosage of a plasmin according to the invention may also depend on the rate of inhibition by endogenous inhibitors such as α2-antiplasmin.

In line with the work herein disclosed, the invention further relates to methods for screening for an autoproteolytically stable plasmin variant, said methods comprising the steps of:

(i) identifying in the catalytic domain of wild-type plasmin at least one internal amino acid at position P of which the peptide bond with internal amino acid at position P+1 is prone to autoproteolysis,

(ii) mutating the amino acid at position P identified in (i) into an amino acid of which the peptide bond with internal amino acid at position P+1 is less or not prone to autoproteolysis,

(iii) determining the autoproteolytic stability of the mutant obtained from (ii), and

(iv) selecting from (iii) a mutant that is autoproteolytically stable as the autoproteolytically stable variant.

The invention likewise relates to methods for screening for an autoproteolytically stable plasmin variant, said methods comprising:

(i) mutating one or more of the arginine or lysine amino acids at positions 137, 147 and 158 of the human plasmin catalytic domain, or of the corresponding arginines or lysines of a non-human plasmin, into an amino acid different from the natural amino acid,

(ii) determining the autoproteolytic stability of the mutant obtained from (i), and

(iii) selecting from (ii) a mutant that is autoproteolytically stable as the autoproteolytically stable plasmin variant;

wherein said human plasmin catalytic domain is starting with the amino acid valine at position 1 which is the same valine amino acid occurring at position 562 of human Glu-plasminogen.

The above screening methods may further comprise a step wherein the proteolytic activity of the autoproteolytically stable plasmin variant is determined.

Many products including medicines (here to be understood specifically as user-ready active ingredient, i.e. in the final formulation for administration to a patient) and bulk-stored active ingredients of medicines are usually stored for a considerable amount of time prior to use. It is of interest to extend the shelf-life of products as long as possible. With the shelf-life is meant the time during which the product can be used safely and during which the product retains it potent utility, i.e. its activity in the case of a medicine and/or its active ingredient. Typically, the shelf-life is indicated on a product or its package. Once the shelf-life has expired, the safe and potent utility of a product is no longer guaranteed. A further important aspect in storing products is the storage temperature at which the desired shelf-life can be achieved. For example, the shelf-life of a product stored at +4° C. or average refrigerator temperature may amount to 12 months whereas the shelf-life of the same product stored at −20° C. or average freezer temperature may amount to 36 months. Logistically, however, maintaining a cold chain at freezing temperatures, e.g. −20° C., is much more complex, difficult and expensive than maintaining a cold chain at +4° C. Thus, it may still be attractive to have a shorter, but sufficiently long shelf-life combined with the possibility to store a product at +4° C. The present invention offers a solution for extending, enhancing or increasing the shelf-life or long-term storage stability of plasmin or any active fragment or derivative thereof or of a composition comprising plasmin or any active derivative thereof. The solution resides in making available plasmin variants as herein described, said variants having an enhanced stability, which, intrinsically, increases, enhances or extends their shelf-life.

The invention likewise relates to methods for enhancing long-term storage stability of a plasmin-comprising composition, said methods comprising the step of identifying an autoproteolytically stable plasmin variant capable of being stored over a long time without significant loss of proteolytic activity. For determining long-term stability, a plasmin preparation according to the invention is aliquoted and activity measurements are performed repeatedly during the envisaged storage term. If the envisaged storage term is, e.g., 24 months, activity measurements can be performed, e.g. every month. The allowable loss of proteolytic activity at the end of the envisaged storage term will largely depend on the envisaged clinical application but typically may be no more than e.g. 10% to 15%.

The invention furthermore relates to methods for producing a plasminogen variant according to the invention, i.e. for producing a plasminogen comprising in its catalytic domain the mutation of at least one internal amino acid at position P of which the peptide bond with internal amino acid at position P+1 is prone to autoproteolysis into an amino acid of which the peptide bond with internal amino acid at position P+1 is less or not prone to autoproteolysis. Such methods include the steps of:

(i) introducing in a suitable host cell a nucleic acid encoding a plasminogen variant according to the invention in a suitable host cell capable of expressing said plasminogen;

(ii) growing the host cell obtained in (i) under conditions and during a time sufficient for expression of said plasminogen in said host cell; and

(iii) harvesting the plasminogen expressed in (ii).

Eventually a step (iv) can be added to such methods which includes the purification of the plasminogen harvested in (iii).

Suitable host cells and methods for expression and production are disclosed in e.g. WO 90/13640 (insect cells), WO 2002/050290 and WO 03/066842 (yeast cells), WO 2008/054592 (bacterial cells/refolding process) and WO 2005/078109 (duckweed transgenic plants or transgenic plant cells).

The invention further encompasses methods for producing a plasmin variant according to the invention, i.e. for producing a plasmin comprising in its catalytic domain the mutation of at least one internal amino acid at position P of which the peptide bond with internal amino acid at position P+1 is prone to autoproteolysis into an amino acid of which the peptide bond with internal amino acid at position P+1 is less or not prone to autoproteolysis. Such methods generally include the steps of producing a plasminogen according to the invention as described above and further comprise a step of activating the plasminogen according to the invention to a plasmin according to the invention using a suitable plasminogen activator (such as tPA, uPA, urokinase, streptokinase, staphylokinase or any variant thereof). Eventually one or more steps can be added wherein the plasminogen is purified prior to activation, activated plasmin is purified and/or active plasmin is derivatized as described above and/or reversibly inactivated and/or, optionally, brought to suitable storage conditions (such as stabilizing solution, lyophilized and/or low temperature).

The invention also relates to (an) isolated nucleic acid sequence(s) encoding a plasminogen variant or plasmin variant according to the invention. The invention also relates to (a) recombinant vector(s) comprising such nucleic acid. The invention also relates to (a) host cell(s) transformed with such nucleic acid or with such recombinant vector.

EXAMPLES

Example 1

Autodegradation of the Plasmin Catalytic Domain and Determination of Peptide Bonds in the Plasmin Catalytic Domain which are Sensitive to Autoproteolysis

In order to study the mechanisms underlying the auto-inactivation of the proteolytic activity of plasmin, the inventor chose to focus on microplasmin which consists mainly of the catalytic domain of plasmin.

A typical size exclusion chromatography (SEC) profile of large-scale produced microplasmin is shown in FIG. **2**. The eluates corresponding to fraction number 5 (pre-peak 1), fraction numbers 7&8 (pre-peak 2), fraction numbers 10-12 (microplasmin peak), and fraction numbers 15&16 (post-peak) were collected and the material therein subjected to N-terminal amino acid sequencing (Edman degradation). The peak eluting around fraction numbers 17-18 corresponds to the buffer peak. SEC was performed on an Amersham Bioscience Superdex 75 10/300 GL column connected to a Waters Alliance HPLC system. The column was equilibrated and eluted with a buffer containing 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 3 mM KCl, 0.5 M $(NH_4)_2SO_4$, pH 7.4. Fifty μL of a 1 mg/mL microplasmin solution (i.e., 50 μg microplasmin) was injected. The eluate was monitored for proteins with UV absorbance detector at 220 nm.

The obtained amino acid sequences are given in Table 2 and correspond to the microplasmin "heavy chain" (starting with amino acids APS, i.e., the 19 C-terminal amino acids of the heavy chain) and light chain (starting with amino acids VVG), and corresponding to two autodegradation products (starting with amino acids EAQ and amino acids VCN). See FIG. **1** for the complete sequence of plasmin(ogen) and indication of heavy- and light-chains and autocleavage sites. The autodegradation products correspond to cleavage of the amide bond C-terminal of Lys 137 and Lys 147, respectively (numbering starting with Val at position 1 of the light chain of plasmin, see FIG. **1**).

TABLE 2

N-terminal amino acid sequences of microplasmin and microplasmin autocatalytic degradation products.

SEC-peak	Sequence 1	2	3	4	5	6	7	8	9	10	SEQ ID NO:
pre-peak 1	A	P	D	F	D X	(C)	G	K	P	Q	43
21.9 mins	V	V	G	G X	(C)	V	A	H	P		44
pre-peak 2	A	P	S	F	D X	(C)	G	K	P	Q	43
24.4 mins	V	V	G	G X	(C)	V	A	H	P		44
	E	**A**	**Q**	**L**	**P**	**V**	**I**	**E**	**N**	**K**	45
	VX	**(C)**	**N**	**R**	**Y**	**E**	**F**	**L**	**N**	**G**	46
μP1 peak	A	P	S	F	D X	(C)	G	K	P	Q	43
27.4 mins	V	V	G	G X	(C)	V	A	H	P	H	44

TABLE 2-continued

N-terminal amino acid sequences of microplasmin and microplasmin autocatalytic degradation products.

SEC-peak	Sequence 1	2	3	4	5	6	7	8	9	10	SEQ ID NO:
post-peak 32.7 mins	**E**	**A**	**Q**	**L**	**P**	**V**	**I**	**E**	**N**	**K**	45

Microplasmin from large-scale production was subjected to autocatalytic degradation. Microplasmin at a final concentration of 0.6 mg/mL was incubated for 4 hrs at +20° C. at pH 3.1, pH 4.0, pH 5.0, pH 6.0, and pH 7.0 after which the samples were immediately frozen at −70° C. The samples were analyzed by reducing SDS-PAGE, the results of which are shown in FIG. **3** (Coomassie Brilliant Blue stained gel). FIG. **3** illustrates major autocatalytic degradation products of about 15 kDa, about 10 kDa and somewhat smaller than 10 kDa. The observed bands are in agreement with cleavage sites as determined via N-terminal amino acid sequencing (see Table 1).

In another set of experiments, large-scale produced microplasmin (4 mg/mL in 5 mM citric acid, 6 mg/mL mannitol, pH 3,1) was diluted in a neutral-pH buffer, and aliquots collected after various times were analyzed either by SDS-PAGE or western blot. For the SDS-PAGE analysis, the data were obtained by diluting microplasmin in BSS+ (Alcon; containing per mL 7.14 mg NaCl, 0.38 mg KCl, 0.154 mg $CaCl_2$, 0.2 mg $MgCl_2$, 0.42 mg Na-phosphate, 2.1 mg $NaHCO_3$, 0.92 mg glucose and 0.184 mg glutathione disulfide; pH 7.4) at a final concentration of 1.25 mg/mL, with the sample kept at room temperature (FIG. **4**A). For the western-blot analysis, microplasmin (final concentration 1.53 μM) was diluted in PBS and incubated at 37° C., and the western blot was developed with a murine anti-microplasmin antibody (FIG. **4**B). FIGS. **4**A and **4**B illustrate the time-dependent degradation of the intact microplasmin and the accumulation of autocatalytic degradation products. Another sample was prepared by diluting the large-scale produced microplasmin 2-fold in 100 mM sodium phosphate, pH 7.2, and the sample was incubated for 30 min at 37° C. Twenty five micrograms of protein were then resolved on a 4-12% polyacrylamide gel. Following Coomassie staining, the bands corresponding to the two degradation fragments were excised, and the peptides were isolated from the gel and submitted to N-terminal sequencing (performed by Eurosequence B.V., Groningen, The Netherlands). The 15 kDa band yielded the sequence expected for the intact catalytic domain (Val-Val-Gly-Gly) (SEQ ID NO: 47). The smaller, 10 kDa fragment yielded the sequence Val-Gln-Ser-Thr-Glu-Leu (SEQ ID NO: 48), which identifies the major cleavage site as being between Arg 158 and Val 159. The 10 kDa fragment also yielded a less abundant (<10%), less well resolved sequence (Xaa-Xaa-Asn-Arg-Tyr), which suggests that a minor cleavage site is located C-terminal to Lys 147. All numberings are starting with Val at position 1 of the light chain of plasmin (see FIG. **1**). Thus, when subjecting microplasmin to autodegradation at 2 mg/mL, an additional autocatalytic cleavage site between Arg 158 and Val 159 was identified.

As is illustrated in FIG. **5**, the kinetics of microplasmin autolysis as assessed by western-blot (circles) follows the loss of microplasmin activity (squares) as assessed by a chromogenic substrate assay (see Example 3). Autolysis data were from the quantification of the band corresponding to the intact microplasmin in FIG. 4B, and from activity data (which were best fitted using a second-order process equation; not shown). From the above described experiments it was concluded that microplasmin autodegradation is responsible for loss of activity, and that the major sites prone to autocatalytic cleavage are between Arg 158 and Val 159, between Lys 147 and Val 148, and between Lys 137 and Glu 138.

Interestingly, the kinetics of inactivation of microplasmin in eye vitreous were very similar to those observed in PBS (FIG. 6A), and western-blot analysis shows that inactivation of microplasmin in eye vitreous also occurs via autolysis (FIG. 6B). For this, microplasmin was diluted in PBS (squares in FIG. 6A) or in porcine eye vitreous (circles in FIG. 6A) to a final concentration of 1.53 μM, and residual concentration of active microplasmin was measured at various time points using the chromogenic substrate Glu-Phe-Lys-pNA. Porcine eye vitreous samples were collected at the indicated times and analyzed by western blot (FIG. 6B) as described above. The arrow indicates the 15-kDa fragment.

Example 2

Construction, Expression and Purification of Plasminogen Variants and Activation to Plasmin

Expression Vector

The pPICZαA secretion vector purchased from Invitrogen Corporation (Carlsbad, Calif.) was used to direct expression and secretion of recombinant human microplasminogen in *Pichia pastoris.*

This vector contains the secretion signal of the *Saccharomyces cerevisiae* α-factor prepropeptide. A XhoI recognition sequence is present at the COOH-terminus of the α-factor secretion signal, immediately upstream of the Lys-Arg site that is cleaved by Kex2 to remove the secretion signal from the mature protein. This XhoI restriction site may be used to clone the gene of interest flush with the Kex2 cleavage site by synthesizing the gene with the XhoI and Kex2 recognition sites at its 5' end. The recombinant gene of interest will then be expressed with the native NH_2-terminus. Engineered immediately downstream from the α-factor secretion signal in the pPICZαA vector is a multiple cloning site with recognition sites for the restriction enzymes EcoRI, SfiI, KpnI, SacII and XbaI to facilitate the cloning of heterologous genes.

Gene Synthesis

To improve expression of human microplasminogen in *Pichia pastoris*, genes encoding the human microplasminogen and variants thereof were synthesized de novo taking into account the preferred codon usage by *Pichia pastoris.*

To design the codon-optimized gene sequence, the human microplasminogen amino acid sequence (SEQ ID NO:2) was imported in the program Gene Designer which is developed by DNA2.0 (Menlo Park, Calif.) and is freely available on the internet. This sequence was backtranslated into DNA sequence using the *Pichia pastoris* codon usage table provided with the program. The nucleotide sequence was then checked manually and adjusted to better fit *Escherichia coli* codon usage. In addition, 6-base pair palindromic sequences and nucleotide repetitions were removed when possible. At the 5' end, an XhoI restriction site and the Kex2 cleavage site were added and at the 3' end, an XbaI restriction site was added.

Mutations were introduced in this wild-type microplasminogen sequence in order to change amino acid residues identified as described in Example 1. Adjacent nucleotides were also changed to introduce a unique restriction site, but in this case care was taken to conserve the encoded amino acid sequence.

In a first variant, the lysine at position 137 is substituted by a glutamine. Lys137 is encoded by the codon AAA at positions 478-480. The nucleotides TTGAAA (positions 475-480) were changed into CTGCAG, introducing a PstI site and changing Lys137 into Gln in the microplasminogen protein, while leaving leucine at position 136 unchanged (nucleotide sequence is in SEQ ID NO:4 and the deduced amino acid sequence in SEQ ID NO:5).

In a second variant, the lysine at position 147 is substituted by a histidine. Lys147 is encoded by the codon AAG at positions 508-510. The nucleotides AAGGTT (positions 508-513) were changed into CACGTG, introducing a Pm1I site and changing Lys147 into H is in the microplasminogen protein, while leaving valine at position 148 unchanged (nucleotide sequence is in SEQ ID NO:6 and the deduced amino acid sequence in SEQ ID NO:7).

In the third variant, the arginine at position 158 is substituted by a histidine. Arg158 is encoded by the codon CGT at positions 540-542. The nucleotides TCGTGTT (positions 539-545) were changed into ACACGTG, introducing a Pm1I site and changing Arg158 into H is in the microplasminogen protein, while leaving glycine at position 157 and valine at position 159 unchanged (nucleotide sequence is in SEQ ID NO:8 and the deduced amino acid sequence in SEQ ID NO:9).

In the fourth variant, all of the changes described above are combined substituting lysine at position 137 by glutamine, lysine at position 147 by histidine and arginine at position 158 by histidine (nucleotide sequence is in SEQ ID NO:10 and the deduced amino acid sequence in SEQ ID NO:11).

Microplasminogen variant sequences were synthesized de novo and cloned into the vector pUC57 by Integrated DNA Technologies (Coralville, Iowa).

In other cases, microplasminogen sequences were synthesized and cloned into the vector pPICZαA by DNA2.0 (Menko Park, Calif.) using the same cloning strategy.

In yet other cases, microplasminogen variants were obtained after site-directed mutagenesis on expression vectors made as described above using the QuikChange II Site Directed Mutagenesis Kit from Stratagene (La Jolla, Calif.). The following primers were used:

```
Lys137Gln mutation:
                                   (sense; SEQ ID NO: 12)
CGTTCGGTGCTGGTCTGCTGCAGGAAGCACAATTACCTGTG
and

                               (antisense; SEQ ID NO: 13)
CACAGGTAATTGTGCTTCCTGCAGCAGACCAGCACCGAACG

Lys137Arg mutation:
                                   (sense; SEQ ID NO: 14)
GGTACGTTCGGTGCTGGTCTGTTGCGTGAAGCACAATTACCTGTGAT
TG
and

                               (antisense; SEQ ID NO: 15)
CAATCACAGGTAATTGTGCTTCACGCAACAGACCAGCACCGAACGTA
CC

Lys147Ala mutation:
                                   (sense; SEQ ID NO: 16)
CAATTACCTGTGATTGAGAACGCCGTGTGTAACAGATACGAGTTC
and

                               (antisense; SEQ ID NO: 17)
GAACTCGTATCTGTTACACACGGCGTTCTCAATCACAGGTAATTG
```

-continued

Lys147Glu mutation:

(sense; SEQ ID NO: 18)
CAATTACCTGTGATTGAGAACGAAGTGTGTAACAGATACGAGTTC
and

(antisense; SEQ ID NO: 19)
GAACTCGTATCTGTTACACACTTCGTTCTCAATCACAGGTAATTG

Lys147Gln mutation:

(sense; SEQ ID NO: 20)
CAATTACCTGTGATTGAGAACCAAGTGTGTAACAGATACGAGTTC
and

(antisense; SEQ ID NO: 21)
GAACTCGTATCTGTTACACACTTGGTTCTCAATCACAGGTAATTG

Arg158Ala mutation:

(sense; SEQ ID NO: 22)
CAGATACGAGTTCCTGAATGGCGCCGTGCAGTCCACTGAGTTGTGTG
CAGG
and

(antisense; SEQ ID NO: 23)
CCTGCACACAACTCAGTGGACTGCACGGCGCCATTCAGGAACTCGTA
TCTG

Arg158Gln mutation:

(sense; SEQ ID NO: 24)
GATACGAGTTCCTGAATGGTCAGGTTCAGTCCACTGAGTTGTGTG
and

(antisense; SEQ ID NO: 25)
CACACAACTCAGTGGACTGAACCTGACCATTCAGGAACTCGTATC

A full list of the single, double and triple mutants made is given in Table 3 (see further).

Expression Vector Construction for Microplasminogen Variants

Wild-type and variant microplasminogen sequences were digested from the vector pUC57 with XhoI and XbaI, and directionally cloned into the vector pPICZαA. The recipient vector-fragment was prepared by XhoI and XbaI restriction and purified from agarose gel using the Qiaquick gel extraction kit (Qiagen GmbH, Germany) The *E. coli* strain TOP10 (Invitrogen) was transformed with the ligation mixture and ampicillin resistant clones were selected. Based on restriction analysis, a plasmid clone containing an insert of the expected size was retained for further characterization. Sequence determination of the resulting plasmid clones confirmed the precise insertion of the microplasminogen coding region fused to the α-factor mating signal, as well as the absence of unwanted mutations in the coding region.

Expression of Microplasminogen Variants and Activation to Plasmin

The microplasminogen variants and activated microplasmin variants are obtained by following essentially the procedure as outlined in Example 2 of WO 02/50290.

Prior to activation, the microplasminogen mutants were purified by immuno-affinity directly from the *Pichia pastoris* supernatants. A murine anti-human microplasmin antibody (raised in Balb/c mice using microplasmin as antigen; produced by hybridoma cell line 7H11A11, available at ThromboGenics N.V.) was coupled on sepharose beads according to the protocol n° 71500015AD from GE Healthcare. Following this protocol, 7.5 mL of immuno-affinity resin were prepared from 45 mg of antibody and packed in a XK 16/20 column. Crude supernatant 200-400 mL (0.2μ filtered from *Pichia* culture/pH 6.0) was directly loaded on the 7H11A11 affinity column After a wash step (100 mM KH_2PO_4, 0.5M NaCl, pH 6.2, 10 column volumes), the microplasminogen variant was eluted with a 0.2M Glycine-HCl, pH 3.0 buffer.

The eluate (fractions 4-6) was neutralized and dialyzed against 25 mM Sodium Phosphate buffer, pH 7.2). The purification of the Lys157Met (K157M) mutant is illustrated in FIG. 7 by means of a chromatogram obtained upon immunoaffinity chromatography (A) and the different eluate fractions were analyzed by SDS-PAGE followed by Coomassie staining (B).

Amino acid sequences and nucleotide sequences of the above described wild-type and variant microplasminogen species are listed hereafter.

SEQ ID NO: 2-Wild-type Human microplasminogen amino acid sequence
APSFDCGKPQVEPKKCPGRVVGGCVAHPHSWPWQVSLRTRFGMHFC
GGTLISPEWVLTAAHCLEKSPRPSSYKVILGAHQEVNLEPHVQEIE
VSRLFLEPTRKDIALLKLSSPAVITDKVIPACLPSPNYVVADRTEC
FITGWGETQGTFGAGLLKEAQLPVIENKVCNRYEFLNGRVQSTELC
AGHLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPG
VYVRVSRFVTWIEGVMRNN

SEQ ID NO: 3-Artificial nucleic acid sequence with optimized codon usage for expression in Pichia. The nucleic acid sequence encodes the wild-type human microplasminogen amino acid sequence of SEQ ID NO: 2
GCACCTTCATTCGACTGTGGTAAGCCTCAGGTCGAACCTAAGAAGT
GTCCAGGTCGTGTTGTCGGTGGCTGTGTGGCTCATCCTCATTCTTG
GCCTTGGCAAGTGTCTCTTAGAACTAGATTTGGTATGCACTTCTGT
GGTGGCACCTTGATCTCACCTGAATGGGTCTTAACCGCAGCTCATT
GTCTGGAGAAGTCACCACGTCCATCTTCATACAAGGTCATCCTTGG
CGCACATCAGGAAGTCAATCTTGAGCCTCATGTTCAGGAGATCGAA
GTCTCTCGTTTGTTCTTGGAACCAACTCGTAAAGACATTGCTCTTC
TGAAGCTGTCATCTCCTGCCGTGATTACCGACAAGGTAATTCCTGC
CTGCTTGCCTAGTCCTAATTACGTCGTTGCCGACCGTACCGAATGC
TTCATTACTGGTTGGGGTGAGACTCAAGGTACGTTCGGTGCTGGTC
TGTTGAAAGAAGCACAATTACCTGTGATTGAGAACAAGGTTTGTAA
CAGATACGAGTTCCTGAATGGTCGTGTTCAGTCCACTGAGTTGTGT
GCAGGTCACCTTGCAGGTGGTACTGATAGTTGTCAAGGTGATTCTG
GTGGACCACTGGTGTGCTTCGAGAAGGATAAGTACATCTTACAAGG
TGTTACGTCTTGGGGTCTTGGATGTGCTCGTCCTAACAAGCCAGGT
GTCTACGTCAGAGTCTCCAGATTCGTAACTTGGATCGAAGGTGTCA
TGCGTAACAACTAA

SEQ ID NO: 4-Microplasminogen variant with the Lys137Gln substitution (mutated codon in bold italics, restriction sites XhoI, PstI and XbaI underlined)
CTCGAGAAAAGAGCACCTTCATTCGACTGTGGTAAGCCTCAGGTCG
AACCTAAGAAGTGTCCAGGTCGTGTTGTCGGTGGCTGTGTGGCTCA
TCCTCATTCTTGGCCTTGGCAAGTGTCTCTTAGAACTAGATTTGGT
ATGCACTTCTGTGGTGGCACCTTGATCTCACCTGAATGGGTCTTAA
CCGCAGCTCATTGTCTGGAGAAGTCACCACGTCCATCTTCATACAA
GGTCATCCTTGGCGCACATCAGGAAGTCAATCTTGAGCCTCATGTT
CAGGAGATCGAAGTCTCTCGTTTGTTCTTGGAACCAACTCGTAAAG
ACATTGCTCTTCTGAAGCTGTCATCTCCTGCCGTGATTACCGACAA
GGTAATTCCTGCCTGCTTGCCTAGTCCTAATTACGTCGTTGCCGAC
CGTACCGAATGCTTCATTACTGGTTGGGGTGAGACTCAAGGTACGT
TCGGTGCTGGTCTGCTG***CAC***GAAGCACAATTACCTGTGATTGAGAA
CAAGGTTTGTAACAGATACGAGTTCCTGAATGGTCGTGTTCAGTCC
ACTGAGTTGTGTGCAGGTCACCTTGCAGGTGGTACTGATAGTTGTC
AAGGTGATTCTGGTGGACCACTGGTGTGCTTCGAGAAGGATAAGTA
CATCTTACAAGGTGTTACGTCTTGGGGTCTTGGATGTGCTCGTCCT
AACAAGCCAGGTGTCTACGTCAGAGTCTCCAGATTCGTAACTTGGA
TCGAAGGTGTCATGCGTAACAACTAATCTAGA

SEQ ID NO: 5-Deduced amino acid sequence of SEQ ID NO: 4 (the underlined N-terminal amino acids "LEKR" (SEQ ID NO: 49) are encoded by the introduced XhoI + Kex2 cleavage sites; the introduced amino acid mutation is indicated in bold/italic and is underlined)
LEKRAPSFDCGKPQVEPKKCPGRVVGGCVAHPHSWPWQVSLRTRFG
MHFCGGTLISPEWVLTAAHCLEKSPRPSSYKVILGAHQEVNLEPHV
QEIEVSRLFLEPTRKDIALLKLSSPAVITDKVIPACLPSPNYVVAD
RTECFITGWGETQGTFGAGLL***Q***EAQLPVIENKVCNRYEFLNGRVQS
TELCAGHLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARP
NKPGVYVRVSRFVTWIEGVMRNN

-continued

SEQ ID NO: 6-Microplasminogen variant with the Lys147His substitution (mutated codon in bold italics, restriction sites XhoI, PmlI and XbaI underlined)

CTCGAGAAAAGAGCACCTTCATTCGACTGTGGTAAGCCTCAGGTCG
AACCTAAGAAGTGTCCAGGTCGTGTTGTCGGTGGCTGTGTGGCTCA
TCCTCATTCTTGGCCTTGGCAAGTGTCTCTTAGAACTAGATTTGGT
ATGCACTTCTGTGGTGGCACCTTGATCTCACCTGAATGGGTCTTAA
CCGCAGCTCATTGTCTGGAGAAGTCACCACGTCCATCTTCATACAA
GGTCATCCTTGGCGCACATCAGGAAGTCAATCTTGAGCCTCATGTT
CAGGAGATCGAAGTCTCTCGTTTGTTCTTGGAACCAACTCGTAAAG
ACATTGCTCTTCTGAAGCTGTCATCTCCTGCCGTGATTACCGACAA
GGTAATTCCTGCCTGCTTGCCTAGTCCTAATTACGTCGTTGCCGAC
CGTACCGAATGCTTCATTACTGGTTGGGGTGAGACTCAAGGTACGT
TCGGTGCTGGTCTGTTGAAAGAAGCACAATTACCTGTGATTGAGAA
C***CAC***GTGTGTAACAGATACGAGTTCCTGAATGGTCGTGTTCAGTCC
ACTGAGTTGTGTGCAGGTCACCTTGCAGGTGGTACTGATAGTTGTC
AAGGTGATTCTGGTGGACCACTGGTGTGCTTCGAGAAGGATAAGTA
CATCTTACAAGGTGTTACGTCTTGGGGTCTTGGATGTGCTCGTCCT
AACAAGCCAGGTGTCTACGTCAGAGTCTCCAGATTCGTAACTTGGA
TCGAAGGTGTCATGCGTAACAACTAATCTAGA

SEQ ID NO: 7-Deduced amino acid sequence of SEQ ID NO: 6 (the underlined N-terminal amino acids "LEKR" (SEQ ID NO: 49) are encoded by the introduced XhoI + Kex2 cleavage sites; the introduced amino acid mutation is indicated in bold/italic and is underlined)

LEKRAPSFDCGKPQVEPKKCPGRVVGGCVAHPHSWPWQVSLRTRFG
MHFCGGTLISPEWVLTAAHCLEKSPRPSSYKVILGAHQEVNLEPHV
QEIEVSRLFLEPTRKDIALLKLSSPAVITDKVIPACLPSPNYVVAD
RTECFITGWGETQGTFGAGLLKEAQLPVIEN***H***VCNRYEFLNGRVQS
TELCAGHLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARP
NKPGVYVRVSRFVTWIEGVMRNN

SEQ ID NO: 8-Microplasminogen variant with the Arg158His substitution (mutated codon in bold italics, restriction sites XhoI, PmlI and XbaI underlined)

CTCGAGAAAAGAGCACCTTCATTCGACTGTGGTAAGCCTCAGGTCG
AACCTAAGAAGTGTCCAGGTCGTGTTGTCGGTGGCTGTGTGGCTCA
TCCTCATTCTTGGCCTTGGCAAGTGTCTCTTAGAACTAGATTTGGT
ATGCACTTCTGTGGTGGCACCTTGATCTCACCTGAATGGGTCTTAA
CCGCAGCTCATTGTCTGGAGAAGTCACCACGTCCATCTTCATACAA
GGTCATCCTTGGCGCACATCAGGAAGTCAATCTTGAGCCTCATGTT
CAGGAGATCGAAGTCTCTCGTTTGTTCTTGGAACCAACTCGTAAAG
ACATTGCTCTTCTGAAGCTGTCATCTCCTGCCGTGATTACCGACAA
GGTAATTCCTGCCTGCTTGCCTAGTCCTAATTACGTCGTTGCCGAC
CGTACCGAATGCTTCATTACTGGTTGGGGTGAGACTCAAGGTACGT
TCGGTGCTGGTCTGTTGAAAGAAGCACAATTACCTGTGATTGAGAA
CAAGGTTTGTAACAGATACGAGTTCCTGAATGGA***CAC***GTGCAGTCC
ACTGAGTTGTGTGCAGGTCACCTTGCAGGTGGTACTGATAGTTGTC
AAGGTGATTCTGGTGGACCACTGGTGTGCTTCGAGAAGGATAAGTA
CATCTTACAAGGTGTTACGTCTTGGGGTCTTGGATGTGCTCGTCCT
AACAAGCCAGGTGTCTACGTCAGAGTCTCCAGATTCGTAACTTGGA
TCGAAGGTGTCATGCGTAACAACTAATCTAGA

SEQ ID NO: 9-Deduced amino acid sequence of SEQ ID NO: 8 (the underlined N-terminal amino acids "LEKR" (SEQ ID NO: 49) are encoded by the introduced XhoI + Kex2 cleavage sites; the introduced amino acid mutation is indicated in bold/italic and is underlined)

LEKRAPSFDCGKPQVEPKKCPGRVVGGCVAHPHSWPWQVSLRTRFG
MHFCGGTLISPEWVLTAAHCLEKSPRPSSYKVILGAHQEVNLEPHV
QEIEVSRLFLEPTRKDIALLKLSSPAVITDKVIPACLPSPNYVVAD
RTECFITGWGETQGTFGAGLLKEAQLPVIENKVCNRYEFLNG***H***VQS
TELCAGHLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARP
PNKPGVYVRVSRFVTWIEGVMRNN

SEQ ID NO: 10-Microplasminogen variant with the Lys137Gln, Lys147His and Arg158His substitutions (mutated codons in bold italics, restriction sites XhoI, PstI, PmlI and XbaI underlined)

CTCGAGAAAAGAGCACCTTCATTCGACTGTGGTAAGCCTCAGGTCG
AACCTAAGAAGTGTCCAGGTCGTGTTGTCGGTGGCTGTGTGGCTCA
TCCTCATTCTTGGCCTTGGCAAGTGTCTCTTAGAACTAGATTTGGT
ATGCACTTCTGTGGTGGCACCTTGATCTCACCTGAATGGGTCTTAA
CCGCAGCTCATTGTCTGGAGAAGTCACCACGTCCATCTTCATACAA
GGTCATCCTTGGCGCACATCAGGAAGTCAATCTTGAGCCTCATGTT
CAGGAGATCGAAGTCTCTCGTTTGTTCTTGGAACCAACTCGTAAAG
ACATTGCTCTTCTGAAGCTGTCATCTCCTGCCGTGATTACCGACAA
GGTAATTCCTGCCTGCTTGCCTAGTCCTAATTACGTCGTTGCCGAC
CGTACCGAATGCTTCATTACTGGTTGGGGTGAGACTCAAGGTACGT
TCGGTGCTGGTCTGCTG***CAG***GAAGCACAATTACCTGTGATTGAGAA
C***CAC***GTGTGTAACAGATACGAGTTCCTGAATGGA***CAC***GTGCAGTCC
ACTGAGTTGTGTGCAGGTCACCTTGCAGGTGGTACTGATAGTTGTC
AAGGTGATTCTGGTGGACCACTGGTGTGCTTCGAGAAGGATAAGTA
CATCTTACAAGGTGTTACGTCTTGGGGTCTTGGATGTGCTCGTCCT
AACAAGCCAGGTGTCTACGTCAGAGTCTCCAGATTCGTAACTTGGA
TCGAAGGTGTCATGCGTAACAACTAATCTAGA

SEQ ID NO: 11-Deduced amino acid sequence of SEQ ID NO: 10 (the underlined N-terminal amino acids "LEKR" (SEQ ID NO: 49) are encoded by the introduced XhoI + Kex2 cleavage sites; the introduced amino acid mutations are indicated in bold/italic and is underlined)

LEKRAPSFDCGKPQVEPKKCPGRVVGGCVAHPHSWPWQVSLRTRFG
MHFCGGTLISPEWVLTAAHCLEKSPRPSSYKVILGAHQEVNLEPHV
QEIEVSRLFLEPTRKDIALLKLSSPAVITDKVIPACLPSPNYVVAD
RTECFITGWGETQGTFGAGLL***Q***EAQLPVIEN***H***VCNRYEFLNG***H***VQS
TELCAGHLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARP
NKPGVYVRVSRFVTWIEGVMRNN

Example 3

Reduced Autoproteolyis of Plasmin Variants Compared to Wild-Type Plasmin

The purified microplasminogen mutants were first converted into the active microplasmin species using recombinant staphylokinase (SAK-SY162) or urokinase (Sigma). Briefly, the microplasminogen mutants (typically 5 to 20 μM in 25 mM sodium phosphate, pH 7.2) were incubated at 37° C. in the presence of staphylokinase (typical microplasminogen/staphylokinase ratio=50/1) or urokinase (typical microplasminogen/urokinase ratio=200), and the appearance of the active microplasmin species was followed by monitoring the hydrolytic activity against the chromogenic substrate S-2403 (used at a concentration of 0.3 mM), as described elsewhere. Once maximal activity was reached, the extent of microplasminogen conversion was assessed by SDS-PAGE and HPLC. Following activation, the autolytic reaction was monitored by measuring the loss of activity in the sample maintained at 37° C. Autolytic degradation was also visualized by SDS-PAGE and HPLC. A typical example of such an experiment is shown in FIGS. **8**A-C. The determination of the second-order rate constant for autolysis (k) was determined as follows: (1) the microplasmin peak area in HPLC was used to calculate the molar concentration of the active microplasmin species (by comparison with a standard curve established with purified, wild-type microplasmin) at the end of the activation phase/beginning of the autolytic phase; (2) the loss of activity measured during the autolytic phase was used to calculate for each time point the residual, molar concentration of active microplasmin; (3) the residual microplasmin concentration (in mol/l) was plotted as a function of time (in s), and the data were fitted with Equation 1 by non-linear regression analysis to obtain an autolysis constant k, the value of which is expressed in $M^{-1}\ s^{-1}$.

$$[\mu PL] = \frac{[\mu PL]_0}{1 + [\mu PL]_0 \cdot k \cdot t} \qquad \text{Equation 1}$$

In Equation 1, [μPL] is the concentration of microplasmin at any given time and $[\mu PL]_0$ is the concentration at t=0. An example of such a curve is shown in FIG. **8**D, and the k values measured for various microplasmin mutants are listed in Table 3 (see further).

SAK-SY162 is a variant of the staphylokinase Sak-STAR (Collen et al. 1992; Fibrinolysis 6, 203-213) with the following amino acid substitutions: K35A, E65Q, K74R, E80A, D82A, T90A, E99D, T101S, E108A, K109A, K130T and K135R.

Example 4

Proteolytic Activity of Plasmin Variants Compared to Wild-Type Plasmin

The hydrolytic activity of microplasmin can be followed using the chromogenic substrate Glu-Phe-Lys-pNA (S-2403, Chromogenix, Milano, Italy). Upon hydrolysis of the substrate, the pNA (p-nitroaniline) group is released, which results in an increase in the absorbance at 405 nm. Activity of wild-type microplasmin and microplasmin variants was measured with the help of a Powerwave X (Bio-Tek) plate reader. Assays were performed at 37° C., in 50 mM Tris, 38 mM NaCl, 0.01% TWEEN-80™, pH 7.4.

For the microplasmin variants, the preparations were first activated with staphylokinase or urokinase, and the concentration of the active microplasmin species was determined at the end of the activation phase as described elsewhere. However, in order to prevent subsequent inactivation, the activated samples were stabilized by lowering the pH to ~3 by addition of 2 volumes of 5 mM citric acid.

The kinetic parameters (k_{cat} & K_m) of the microplasmin variants against the chromogenic substrate S-2403 were obtained by measuring initial rates of hydrolysis at various substrate concentrations, and by analysing the data with Equation 2, where [μPL] is the concentration of active microplasmin as measured by HPLC, and [S] is the concentration of S-2403. An example of k_{cat} and K_m determination from the measurement of initial rates of hydrolysis is shown in FIG. **9**.

$$v_i = \frac{k_{cat} \cdot [\mu PL] \cdot [S]}{K_m + [S]} \qquad \text{Equation 2}$$

The k_{cat} and K_m values obtained for various microplasmin mutants are listed in Table 3.

TABLE 3

Overview of kinetic parameters (k_{cat} and K_m) and autolysis rate constants of wild-type microplasmin and a series of single, double, and triple mutants.

	Kinetic parameters		Autolysis rate constant
Mutant	k_{cat} (s^{-1})	K_m (M)	k ($M^{-1}s^{-1}$)
wild-type	46	7.6×10^{-5}	230
137A	61	1.4×10^{-2}	3
137E	5	2.2×10^{-3}	1
137F	29	4.0×10^{-3}	1.6
137H	54	6.0×10^{-3}	8
137I	ND	ND	5
137M	36	4.7×10^{-3}	1
137Q	55	3.6×10^{-3}	10
137R	39	8.1×10^{-3}	3
147A	34	1.3×10^{-4}	24
147E	35	9.2×10^{-5}	21
147F	32	1.0×10^{-4}	122
147H	51	1.3×10^{-4}	118
147I	36	1.1×10^{-4}	76

TABLE 3-continued

Overview of kinetic parameters (k_{cat} and K_m) and autolysis rate constants of wild-type microplasmin and a series of single, double, and triple mutants.

	Kinetic parameters		Autolysis rate constant
Mutant	k_{cat} (s^{-1})	K_m (M)	k ($M^{-1}s^{-1}$)
147Q	39	8.5×10^{-5}	45
158A	32	1.2×10^{-4}	80
158E	24	1.8×10^{-4}	86
158F	36	2.2×10^{-4}	159
158H	59	1.7×10^{-4}	192
158I	31	2.1×10^{-4}	66
158Q	29	1.2×10^{-4}	59
137A147A	64	1.6×10^{-2}	5
137A147H	40	1.2×10^{-2}	1
137A158A	36	6.4×10^{-3}	1.4
137A158H	30	1.1×10^{-2}	0.7
137H147H	38	6.2×10^{-3}	3
137H158H	40	7.7×10^{-3}	2
137Q147H	69	8×10^{-3}	<0.5
137Q158H	38	3.9×10^{-3}	<1.3
147A158A	33	7.9×10^{-5}	26
147A158H	27	1.1×10^{-4}	57
147H158H	50	1.7×10^{-4}	163
147H158A	29	1.3×10^{-4}	30
137A147A158A	46	8.3×10^{-3}	<0.8
137A147H158H	25	9.1×10^{-3}	<0.7
137H147A158A	27	3.2×10^{-3}	<1.2
137H147H158H	34	4.5×10^{-3}	<0.6
137Q147H158H	45	6.6×10^{-3}	1
137R147H158H	30	7.2×10^{-3}	<4

Example 5

Therapeutic Efficacy of Plasmin Variants in In Vitro or In Vivo Models

5.1 Effect of Plasmin Variants on Cerebral Infarct Size.

The efficacy of the plasmin variants of the invention in reducing cerebral infarct size can be performed in a murine cerebral infarct model such as described in Example 2 of WO 00/18436, or according to Welsh et al. (1987, J Neurochem 49, 846-851). The beneficial effect of wild-type plasmin on cerebral infarct size was demonstrated in Example 5 of WO 00/18436. A similar experiment is performed with any of the plasmin variants of the invention and the beneficial effect of these plasmin variants is measured and compared to the beneficial effect of wild-type plasmin.

5.2 In Vivo Thrombolytic Activity of Plasmin Variants

The rabbit extracorporeal loop thrombolysis model (Example 6 of WO 02/50290; Hotchkiss et al., 1987, Thromb Haemost 58, 107—Abstract 377), the dog circumflex coronary artery copper coil-induced thrombosis model (Example 8 of WO 02/50290; Bergmann et al., 1983, Science 220, 1181-1183) or the rabbit jugular vein thrombosis model (Collen et al., 1983, J Clin Invest 71, 368-376) can be used to demonstrate in vivo thrombolytic activity of the plasmin variants of the invention. The beneficial effect of wild-type plasmin on thrombolysis was demonstrated with these models as described in Examples 7 and 9 of WO 00/18436 and by Collen et al. (1983). Similar experiments are performed with any of the plasmin variants of the invention and the beneficial effect of these plasmin variants is measured and compared to the beneficial effect of wild-type plasmin.

5.3 In Vitro Thrombolytic Activity of Plasmin Variants

An in vitro model of peripheral arterial occlusion (P AO) is described in Example 6 of WO 01/36609 and the thrombolytic efficacy of wild-type plasmin was demonstrated in this model. A similar experiment is performed with any of the plasmin variants of the invention and the beneficial effect of these plasmin variants on thrombolysis of peripheral arterial occlusions is measured and compared to the beneficial effect of wild-type plasmin.

5.4 Liquefaction of Eye Vitreous and Posterior Vitreous Detachment Induced by Plasmin Variants

Example 5 of WO 2004/052228 discloses an assay for determining the efficacy, as well as the efficacy of microplasmin in liquefying the vitreous in post-mortem pig eyes. Example 6 of WO 2004/052228 discloses an assay for determining the efficacy, as well as the efficacy of microplasmin in inducing posterior vitreous detachment (PVD) in human post-mortem eyes. Induction of vitreous liquefaction and PVD by the plasmin variants of the invention is demonstrated in similar post-mortem models.

5.5 In Vivo PVD Induced by Plasmin Variants

Example 7 of WO 2004/052228 discloses an assay for determining the efficacy, as well as the efficacy of microplasmin in inducing PVD in an in vivo feline model. Induction of PVD by the plasmin variants of the invention is demonstrated in a similar in vivo model.

SEQUENCE LISTING

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<160> NUMBER OF SEQ ID NOS: 50

<210> SEQ ID NO 1
<211> LENGTH: 791
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

Glu Pro Leu Asp Asp Tyr Val Asn Thr Gln Gly Ala Ser Leu Phe Ser
1               5                   10                  15

Val Thr Lys Lys Gln Leu Gly Ala Gly Ser Ile Glu Glu Cys Ala Ala
            20                  25                  30

Lys Cys Glu Glu Asp Glu Glu Phe Thr Cys Arg Ala Phe Gln Tyr His
        35                  40                  45

Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Arg Lys Ser Ser
    50                  55                  60

Ile Ile Ile Arg Met Arg Asp Val Val Leu Phe Glu Lys Lys Val Tyr
65                  70                  75                  80

Leu Ser Glu Cys Lys Thr Gly Asn Gly Lys Asn Tyr Arg Gly Thr Met
                85                  90                  95

Ser Lys Thr Lys Asn Gly Ile Thr Cys Gln Lys Trp Ser Ser Thr Ser
            100                 105                 110

Pro His Arg Pro Arg Phe Ser Pro Ala Thr His Pro Ser Glu Gly Leu
        115                 120                 125

Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Pro Gln Gly Pro Trp
    130                 135                 140

Cys Tyr Thr Thr Asp Pro Glu Lys Arg Tyr Asp Tyr Cys Asp Ile Leu
145                 150                 155                 160

Glu Cys Glu Glu Glu Cys Met His Cys Ser Gly Glu Asn Tyr Asp Gly
                165                 170                 175

Lys Ile Ser Lys Thr Met Ser Gly Leu Glu Cys Gln Ala Trp Asp Ser
            180                 185                 190

Gln Ser Pro His Ala His Gly Tyr Ile Pro Ser Lys Phe Pro Asn Lys
        195                 200                 205

Asn Leu Lys Lys Asn Tyr Cys Arg Asn Pro Asp Arg Glu Leu Arg Pro
    210                 215                 220

Trp Cys Phe Thr Thr Asp Pro Asn Lys Arg Trp Glu Leu Cys Asp Ile
225                 230                 235                 240

Pro Arg Cys Thr Thr Pro Pro Pro Ser Ser Gly Pro Thr Tyr Gln Cys
                245                 250                 255

Leu Lys Gly Thr Gly Glu Asn Tyr Arg Gly Asn Val Ala Val Thr Val
            260                 265                 270

Ser Gly His Thr Cys Gln His Trp Ser Ala Gln Thr Pro His Thr His
        275                 280                 285
```

```
Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Asp Glu Asn Tyr
    290                 295                 300

Cys Arg Asn Pro Asp Gly Lys Arg Ala Pro Trp Cys His Thr Thr Asn
305                 310                 315                 320

Ser Gln Val Arg Trp Glu Tyr Cys Lys Ile Pro Ser Cys Asp Ser Ser
                325                 330                 335

Pro Val Ser Thr Glu Gln Leu Ala Pro Thr Ala Pro Pro Glu Leu Thr
            340                 345                 350

Pro Val Val Gln Asp Cys Tyr His Gly Asp Gly Gln Ser Tyr Arg Gly
        355                 360                 365

Thr Ser Ser Thr Thr Thr Thr Gly Lys Lys Cys Gln Ser Trp Ser Ser
    370                 375                 380

Met Thr Pro His Arg His Gln Lys Thr Pro Glu Asn Tyr Pro Asn Ala
385                 390                 395                 400

Gly Leu Thr Met Asn Tyr Cys Arg Asn Pro Asp Ala Asp Lys Gly Pro
                405                 410                 415

Trp Cys Phe Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr Cys Asn Leu
            420                 425                 430

Lys Lys Cys Ser Gly Thr Glu Ala Ser Val Val Ala Pro Pro Pro Val
        435                 440                 445

Val Leu Leu Pro Asp Val Glu Thr Pro Ser Glu Glu Asp Cys Met Phe
    450                 455                 460

Gly Asn Gly Lys Gly Tyr Arg Gly Lys Arg Ala Thr Thr Val Thr Gly
465                 470                 475                 480

Thr Pro Cys Gln Asp Trp Ala Ala Gln Glu Pro His Arg His Ser Ile
                485                 490                 495

Phe Thr Pro Glu Thr Asn Pro Arg Ala Gly Leu Glu Lys Asn Tyr Cys
            500                 505                 510

Arg Asn Pro Asp Gly Asp Val Gly Gly Pro Trp Cys Tyr Thr Thr Asn
        515                 520                 525

Pro Arg Lys Leu Tyr Asp Tyr Cys Asp Val Pro Gln Cys Ala Ala Pro
    530                 535                 540

Ser Phe Asp Cys Gly Lys Pro Gln Val Glu Pro Lys Lys Cys Pro Gly
545                 550                 555                 560

Arg Val Val Gly Gly Cys Val Ala His Pro His Ser Trp Pro Trp Gln
                565                 570                 575

Val Ser Leu Arg Thr Arg Phe Gly Met His Phe Cys Gly Gly Thr Leu
            580                 585                 590

Ile Ser Pro Glu Trp Val Leu Thr Ala Ala His Cys Leu Glu Lys Ser
        595                 600                 605

Pro Arg Pro Ser Ser Tyr Lys Val Ile Leu Gly Ala His Gln Glu Val
    610                 615                 620

Asn Leu Glu Pro His Val Gln Glu Ile Glu Val Ser Arg Leu Phe Leu
625                 630                 635                 640

Glu Pro Thr Arg Lys Asp Ile Ala Leu Leu Lys Leu Ser Ser Pro Ala
                645                 650                 655

Val Ile Thr Asp Lys Val Ile Pro Ala Cys Leu Pro Ser Pro Asn Tyr
            660                 665                 670

Val Val Ala Asp Arg Thr Glu Cys Phe Ile Thr Gly Trp Gly Glu Thr
        675                 680                 685

Gln Gly Thr Phe Gly Ala Gly Leu Leu Lys Glu Ala Gln Leu Pro Val
    690                 695                 700

Ile Glu Asn Lys Val Cys Asn Arg Tyr Glu Phe Leu Asn Gly Arg Val
```

```
705                 710                 715                 720

Gln Ser Thr Glu Leu Cys Ala Gly His Leu Ala Gly Gly Thr Asp Ser
                725                 730                 735

Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Phe Glu Lys Asp Lys
            740                 745                 750

Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu Gly Cys Ala Arg Pro
        755                 760                 765

Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg Phe Val Thr Trp Ile
    770                 775                 780

Glu Gly Val Met Arg Asn Asn
785                 790


<210> SEQ ID NO 2
<211> LENGTH: 249
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

Ala Pro Ser Phe Asp Cys Gly Lys Pro Gln Val Glu Pro Lys Lys Cys
1               5                   10                  15

Pro Gly Arg Val Val Gly Gly Cys Val Ala His Pro His Ser Trp Pro
            20                  25                  30

Trp Gln Val Ser Leu Arg Thr Arg Phe Gly Met His Phe Cys Gly Gly
        35                  40                  45

Thr Leu Ile Ser Pro Glu Trp Val Leu Thr Ala Ala His Cys Leu Glu
    50                  55                  60

Lys Ser Pro Arg Pro Ser Ser Tyr Lys Val Ile Leu Gly Ala His Gln
65                  70                  75                  80

Glu Val Asn Leu Glu Pro His Val Gln Glu Ile Glu Val Ser Arg Leu
                85                  90                  95

Phe Leu Glu Pro Thr Arg Lys Asp Ile Ala Leu Leu Lys Leu Ser Ser
            100                 105                 110

Pro Ala Val Ile Thr Asp Lys Val Ile Pro Ala Cys Leu Pro Ser Pro
        115                 120                 125

Asn Tyr Val Val Ala Asp Arg Thr Glu Cys Phe Ile Thr Gly Trp Gly
    130                 135                 140

Glu Thr Gln Gly Thr Phe Gly Ala Gly Leu Leu Lys Glu Ala Gln Leu
145                 150                 155                 160

Pro Val Ile Glu Asn Lys Val Cys Asn Arg Tyr Glu Phe Leu Asn Gly
                165                 170                 175

Arg Val Gln Ser Thr Glu Leu Cys Ala Gly His Leu Ala Gly Gly Thr
            180                 185                 190

Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Phe Glu Lys
        195                 200                 205

Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu Gly Cys Ala
    210                 215                 220

Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg Phe Val Thr
225                 230                 235                 240

Trp Ile Glu Gly Val Met Arg Asn Asn
                245


<210> SEQ ID NO 3
<211> LENGTH: 750
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Artificial sequence encoding wild-type human
      microplasminogen - sequence contains codons optimized for
      expression in Pichia

<400> SEQUENCE: 3

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gcaccttcat tcgactgtgg taagcctcag gtcgaaccta agaagtgtcc aggtcgtgtt     60

gtcggtggct gtgtggctca tcctcattct tggccttggc aagtgtctct tagaactaga    120

tttggtatgc acttctgtgg tggcaccttg atctcacctg aatgggtctt aaccgcagct    180

cattgtctgg agaagtcacc acgtccatct tcatacaagg tcatccttgg cgcacatcag    240

gaagtcaatc ttgagcctca tgttcaggag atcgaagtct ctcgtttgtt cttggaacca    300

actcgtaaag acattgctct tctgaagctg tcatctcctg ccgtgattac cgacaaggta    360

attcctgcct gcttgcctag tcctaattac gtcgttgccg accgtaccga atgcttcatt    420

actggttggg gtgagactca aggtacgttc ggtgctggtc tgttgaaaga agcacaatta    480

cctgtgattg agaacaaggt ttgtaacaga tacgagttcc tgaatggtcg tgttcagtcc    540

actgagttgt gtgcaggtca ccttgcaggt ggtactgata gttgtcaagg tgattctggt    600

ggaccactgg tgtgcttcga gaaggataag tacatcttac aaggtgttac gtcttggggt    660

cttggatgtg ctcgtcctaa caagccaggt gtctacgtca gagtctccag attcgtaact    720

tggatcgaag gtgtcatgcg taacaactaa                                     750
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<210> SEQ ID NO 4
<211> LENGTH: 768
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Artificial sequence encoding variant human
      microplasminogen with K137Q mutation - sequence contains codons
      optimized for expression in Pichia

<400> SEQUENCE: 4

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ctcgagaaaa gagcaccttc attcgactgt ggtaagcctc aggtcgaacc taagaagtgt     60

ccaggtcgtg ttgtcggtgg ctgtgtggct catcctcatt cttggccttg gcaagtgtct    120

cttagaacta gatttggtat gcacttctgt ggtggcacct tgatctcacc tgaatgggtc    180

ttaaccgcag ctcattgtct ggagaagtca ccacgtccat cttcatacaa ggtcatcctt    240

ggcgcacatc aggaagtcaa tcttgagcct catgttcagg agatcgaagt ctctcgtttg    300

ttcttggaac caactcgtaa agacattgct cttctgaagc tgtcatctcc tgccgtgatt    360

accgacaagg taattcctgc ctgcttgcct agtcctaatt acgtcgttgc cgaccgtacc    420

gaatgcttca ttactggttg gggtgagact caaggtacgt tcggtgctgg tctgctgcag    480

gaagcacaat tacctgtgat tgagaacaag gtttgtaaca gatacgagtt cctgaatggt    540

cgtgttcagt ccactgagtt gtgtgcaggt caccttgcag gtggtactga tagttgtcaa    600

ggtgattctg gtggaccact ggtgtgcttc gagaaggata agtacatctt acaaggtgtt    660

acgtcttggg gtcttggatg tgctcgtcct aacaagccag gtgtctacgt cagagtctcc    720

agattcgtaa cttggatcga aggtgtcatg cgtaacaact aatctaga                 768
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<210> SEQ ID NO 5
<211> LENGTH: 253

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: Variant human microplasminogen with K137Q
      mutation and comprising N-terminal sequence LEKR of which KR is a
      KEX2 cleavage site

<400> SEQUENCE: 5

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Leu Glu Lys Arg Ala Pro Ser Phe Asp Cys Gly Lys Pro Gln Val Glu
1               5                   10                  15

Pro Lys Lys Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala His Pro
            20                  25                  30

His Ser Trp Pro Trp Gln Val Ser Leu Arg Thr Arg Phe Gly Met His
        35                  40                  45

Phe Cys Gly Gly Thr Leu Ile Ser Pro Glu Trp Val Leu Thr Ala Ala
    50                  55                  60

His Cys Leu Glu Lys Ser Pro Arg Pro Ser Ser Tyr Lys Val Ile Leu
65                  70                  75                  80

Gly Ala His Gln Glu Val Asn Leu Glu Pro His Val Gln Glu Ile Glu
                85                  90                  95

Val Ser Arg Leu Phe Leu Glu Pro Thr Arg Lys Asp Ile Ala Leu Leu
            100                 105                 110

Lys Leu Ser Ser Pro Ala Val Ile Thr Asp Lys Val Ile Pro Ala Cys
        115                 120                 125

Leu Pro Ser Pro Asn Tyr Val Val Ala Asp Arg Thr Glu Cys Phe Ile
    130                 135                 140

Thr Gly Trp Gly Glu Thr Gln Gly Thr Phe Gly Ala Gly Leu Leu Gln
145                 150                 155                 160

Glu Ala Gln Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Tyr Glu
                165                 170                 175

Phe Leu Asn Gly Arg Val Gln Ser Thr Glu Leu Cys Ala Gly His Leu
            180                 185                 190

Ala Gly Gly Thr Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val
        195                 200                 205

Cys Phe Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly
    210                 215                 220

Leu Gly Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser
225                 230                 235                 240

Arg Phe Val Thr Trp Ile Glu Gly Val Met Arg Asn Asn
                245                 250
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<210> SEQ ID NO 6
<211> LENGTH: 768
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<220> FEATURE:
<223> OTHER INFORMATION: Artificial sequence encoding variant human
      microplasminogen with K147H mutation - sequence contains codons
      optimized for expression in Pichia

<400> SEQUENCE: 6

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ctcgagaaaa gagcaccttc attcgactgt ggtaagcctc aggtcgaacc taagaagtgt     60

ccaggtcgtg ttgtcggtgg ctgtgtggct catcctcatt cttggccttg gcaagtgtct    120
```

```
cttagaacta gatttggtat gcacttctgt ggtggcacct tgatctcacc tgaatgggtc    180

ttaaccgcag ctcattgtct ggagaagtca ccacgtccat cttcatacaa ggtcatcctt    240

ggcgcacatc aggaagtcaa tcttgagcct catgttcagg agatcgaagt ctctcgtttg    300

ttcttggaac caactcgtaa agacattgct cttctgaagc tgtcatctcc tgccgtgatt    360

accgacaagg taattcctgc ctgcttgcct agtcctaatt acgtcgttgc cgaccgtacc    420

gaatgcttca ttactggttg ggtgagact caaggtacgt tcggtgctgg tctgttgaaa    480

gaagcacaat tacctgtgat tgagaaccac gtgtgtaaca gatacgagtt cctgaatggt    540

cgtgttcagt ccactgagtt gtgtgcaggt caccttgcag gtggtactga tagttgtcaa    600

ggtgattctg gtggaccact ggtgtgcttc gagaaggata agtacatctt acaaggtgtt    660

acgtcttggg gtcttggatg tgctcgtcct aacaagccag gtgtctacgt cagagtctcc    720

agattcgtaa cttggatcga aggtgtcatg cgtaacaact aatctaga                 768
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<210> SEQ ID NO 7
<211> LENGTH: 253
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: Variant human microplasminogen with K147H
      mutation and comprising N-terminal sequence LEKR of which KR is a
      KEX2 cleavage site

<400> SEQUENCE: 7

Leu Glu Lys Arg Ala Pro Ser Phe Asp Cys Gly Lys Pro Gln Val Glu
1               5                   10                  15

Pro Lys Lys Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala His Pro
            20                  25                  30

His Ser Trp Pro Trp Gln Val Ser Leu Arg Thr Arg Phe Gly Met His
        35                  40                  45

Phe Cys Gly Gly Thr Leu Ile Ser Pro Glu Trp Val Leu Thr Ala Ala
    50                  55                  60

His Cys Leu Glu Lys Ser Pro Arg Pro Ser Ser Tyr Lys Val Ile Leu
65                  70                  75                  80

Gly Ala His Gln Glu Val Asn Leu Glu Pro His Val Gln Glu Ile Glu
                85                  90                  95

Val Ser Arg Leu Phe Leu Glu Pro Thr Arg Lys Asp Ile Ala Leu Leu
            100                 105                 110

Lys Leu Ser Ser Pro Ala Val Ile Thr Asp Lys Val Ile Pro Ala Cys
        115                 120                 125

Leu Pro Ser Pro Asn Tyr Val Val Ala Asp Arg Thr Glu Cys Phe Ile
    130                 135                 140

Thr Gly Trp Gly Glu Thr Gln Gly Thr Phe Gly Ala Gly Leu Leu Lys
145                 150                 155                 160

Glu Ala Gln Leu Pro Val Ile Glu Asn His Val Cys Asn Arg Tyr Glu
                165                 170                 175

Phe Leu Asn Gly Arg Val Gln Ser Thr Glu Leu Cys Ala Gly His Leu
            180                 185                 190

Ala Gly Gly Thr Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val
        195                 200                 205

Cys Phe Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly
    210                 215                 220
```

```
Leu Gly Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser
225                 230                 235                 240

Arg Phe Val Thr Trp Ile Glu Gly Val Met Arg Asn Asn
                245                 250


<210> SEQ ID NO 8
<211> LENGTH: 768
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Artificial sequence encoding variant human
      microplasminogen with R158H mutation - sequence contains codons
      optimized for expression in Pichia

<400> SEQUENCE: 8

ctcgagaaaa gagcaccttc attcgactgt ggtaagcctc aggtcgaacc taagaagtgt      60

ccaggtcgtg ttgtcggtgg ctgtgtggct catcctcatt cttggccttg gcaagtgtct     120

cttagaacta gatttggtat gcacttctgt ggtggcacct tgatctcacc tgaatgggtc     180

ttaaccgcag ctcattgtct ggagaagtca ccacgtccat cttcatacaa ggtcatcctt     240

ggcgcacatc aggaagtcaa tcttgagcct catgttcagg agatcgaagt ctctcgtttg     300

ttcttggaac caactcgtaa agacattgct cttctgaagc tgtcatctcc tgccgtgatt     360

accgacaagg taattcctgc ctgcttgcct agtcctaatt acgtcgttgc cgaccgtacc     420

gaatgcttca ttactggttg gggtgagact caaggtacgt tcggtgctgg tctgttgaaa     480

gaagcacaat tacctgtgat tgagaacaag gtttgtaaca gatacgagtt cctgaatgga     540

cacgtgcagt ccactgagtt gtgtgcaggt caccttgcag gtggtactga tagttgtcaa     600

ggtgattctg gtggaccact ggtgtgcttc gagaaggata agtacatctt acaaggtgtt     660

acgtcttggg gtcttggatg tgctcgtcct aacaagccag gtgtctacgt cagagtctcc     720

agattcgtaa cttggatcga aggtgtcatg cgtaacaact aatctaga                  768


<210> SEQ ID NO 9
<211> LENGTH: 253
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: Variant human microplasminogen with R158H
      mutation and comprising N-terminal sequence LEKR of which KR is a
      KEX2 cleavage site

<400> SEQUENCE: 9

Leu Glu Lys Arg Ala Pro Ser Phe Asp Cys Gly Lys Pro Gln Val Glu
1               5                   10                  15

Pro Lys Lys Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala His Pro
            20                  25                  30

His Ser Trp Pro Trp Gln Val Ser Leu Arg Thr Arg Phe Gly Met His
        35                  40                  45

Phe Cys Gly Gly Thr Leu Ile Ser Pro Glu Trp Val Leu Thr Ala Ala
    50                  55                  60

His Cys Leu Glu Lys Ser Pro Arg Pro Ser Ser Tyr Lys Val Ile Leu
65                  70                  75                  80

Gly Ala His Gln Glu Val Asn Leu Glu Pro His Val Gln Glu Ile Glu
                85                  90                  95
```

```
Val Ser Arg Leu Phe Leu Glu Pro Thr Arg Lys Asp Ile Ala Leu Leu
            100                 105                 110

Lys Leu Ser Ser Pro Ala Val Ile Thr Asp Lys Val Ile Pro Ala Cys
        115                 120                 125

Leu Pro Ser Pro Asn Tyr Val Val Ala Asp Arg Thr Glu Cys Phe Ile
    130                 135                 140

Thr Gly Trp Gly Glu Thr Gln Gly Thr Phe Gly Ala Gly Leu Leu Lys
145                 150                 155                 160

Glu Ala Gln Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Tyr Glu
                165                 170                 175

Phe Leu Asn Gly His Val Gln Ser Thr Glu Leu Cys Ala Gly His Leu
            180                 185                 190

Ala Gly Gly Thr Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val
        195                 200                 205

Cys Phe Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly
    210                 215                 220

Leu Gly Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser
225                 230                 235                 240

Arg Phe Val Thr Trp Ile Glu Gly Val Met Arg Asn Asn
                245                 250
```

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<210> SEQ ID NO 10
<211> LENGTH: 768
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Artificial sequence encoding variant human
      microplasminogen with K137Q, K147H and R158H mutations - sequence
      contains codons optimized for expression in Pichia

<400> SEQUENCE: 10

ctcgagaaaa gagcaccttc attcgactgt ggtaagcctc aggtcgaacc taagaagtgt      60

ccaggtcgtg ttgtcggtgg ctgtgtggct catcctcatt cttggccttg gcaagtgtct     120

cttagaacta gatttggtat gcacttctgt ggtggcacct tgatctcacc tgaatgggtc     180

ttaaccgcag ctcattgtct ggagaagtca ccacgtccat cttcatacaa ggtcatcctt     240

ggcgcacatc aggaagtcaa tcttgagcct catgttcagg agatcgaagt ctctcgtttg     300

ttcttggaac caactcgtaa agacattgct cttctgaagc tgtcatctcc tgccgtgatt     360

accgacaagg taattcctgc ctgcttgcct agtcctaatt acgtcgttgc cgaccgtacc     420

gaatgcttca ttactggttg gggtgagact caaggtacgt tcggtgctgg tctgctgcag     480

gaagcacaat tacctgtgat tgagaaccac gtgtgtaaca gatacgagtt cctgaatgga     540

cacgtgcagt ccactgagtt gtgtgcaggt caccttgcag gtggtactga tagttgtcaa     600

ggtgattctg gtggaccact ggtgtgcttc gagaaggata agtacatctt acaaggtgtt     660

acgtcttggg gtcttggatg tgctcgtcct aacaagccag gtgtctacgt cagagtctcc     720

agattcgtaa cttggatcga aggtgtcatg cgtaacaact aatctaga                  768


<210> SEQ ID NO 11
<211> LENGTH: 253
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
```

```
      polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: Variant human microplasminogen with K137Q,
      K147H and R158H mutations and comprising N-terminal sequence LEKR
      of which KR is a KEX2 cleavage site

<400> SEQUENCE: 11

Leu Glu Lys Arg Ala Pro Ser Phe Asp Cys Gly Lys Pro Gln Val Glu
1               5                   10                  15

Pro Lys Lys Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala His Pro
            20                  25                  30

His Ser Trp Pro Trp Gln Val Ser Leu Arg Thr Arg Phe Gly Met His
        35                  40                  45

Phe Cys Gly Gly Thr Leu Ile Ser Pro Glu Trp Val Leu Thr Ala Ala
    50                  55                  60

His Cys Leu Glu Lys Ser Pro Arg Pro Ser Ser Tyr Lys Val Ile Leu
65                  70                  75                  80

Gly Ala His Gln Glu Val Asn Leu Glu Pro His Val Gln Glu Ile Glu
                85                  90                  95

Val Ser Arg Leu Phe Leu Glu Pro Thr Arg Lys Asp Ile Ala Leu Leu
            100                 105                 110

Lys Leu Ser Ser Pro Ala Val Ile Thr Asp Lys Val Ile Pro Ala Cys
        115                 120                 125

Leu Pro Ser Pro Asn Tyr Val Val Ala Asp Arg Thr Glu Cys Phe Ile
    130                 135                 140

Thr Gly Trp Gly Glu Thr Gln Gly Thr Phe Gly Ala Gly Leu Leu Gln
145                 150                 155                 160

Glu Ala Gln Leu Pro Val Ile Glu Asn His Val Cys Asn Arg Tyr Glu
                165                 170                 175

Phe Leu Asn Gly His Val Gln Ser Thr Glu Leu Cys Ala Gly His Leu
            180                 185                 190

Ala Gly Gly Thr Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val
        195                 200                 205

Cys Phe Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly
    210                 215                 220

Leu Gly Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser
225                 230                 235                 240

Arg Phe Val Thr Trp Ile Glu Gly Val Met Arg Asn Asn
                245                 250


<210> SEQ ID NO 12
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      sense oligonucleotide Lys137Gln mutation

<400> SEQUENCE: 12

cgttcggtgc tggtctgctg caggaagcac aattacctgt g                          41


<210> SEQ ID NO 13
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      antisense oligonucleotide Lys137Gln mutation

<400> SEQUENCE: 13
```

```
cacaggtaat tgtgcttcct gcagcagacc agcaccgaac g                     41


<210> SEQ ID NO 14
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      sense oligonucleotide Lys137Arg mutation

<400> SEQUENCE: 14

ggtacgttcg gtgctggtct gttgcgtgaa gcacaattac ctgtgattg             49


<210> SEQ ID NO 15
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      antisense oligonucleotide Lys137Arg mutation

<400> SEQUENCE: 15

caatcacagg taattgtgct tcacgcaaca gaccagcacc gaacgtacc             49


<210> SEQ ID NO 16
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      sense oligonucleotide Lys147Ala mutation

<400> SEQUENCE: 16

caattacctg tgattgagaa cgccgtgtgt aacagatacg agttc                 45


<210> SEQ ID NO 17
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      antisense oligonucleotide Lys147Ala mutation

<400> SEQUENCE: 17

gaactcgtat ctgttacaca cggcgttctc aatcacaggt aattg                 45


<210> SEQ ID NO 18
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      sense oligonucleotide Lys147Glu mutation

<400> SEQUENCE: 18

caattacctg tgattgagaa cgaagtgtgt aacagatacg agttc                 45


<210> SEQ ID NO 19
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      antisense oligonucleotide Lys147Glu mutation

<400> SEQUENCE: 19
```

gaactcgtat ctgttacaca cttcgttctc aatcacaggt aattg 45

<210> SEQ ID NO 20
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
sense oligonucleotide Lys147Gln mutation

<400> SEQUENCE: 20

caattacctg tgattgagaa ccaagtgtgt aacagatacg agttc 45

<210> SEQ ID NO 21
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
antisense oligonucleotide Lys147Gln mutation

<400> SEQUENCE: 21

gaactcgtat ctgttacaca cttggttctc aatcacaggt aattg 45

<210> SEQ ID NO 22
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
sense oligonucleotide Arg158Ala mutation

<400> SEQUENCE: 22

cagatacgag ttcctgaatg gcgccgtgca gtccactgag ttgtgtgcag g 51

<210> SEQ ID NO 23
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
antisense oligonucleotide Arg158Ala mutation

<400> SEQUENCE: 23

cctgcacaca actcagtgga ctgcacggcg ccattcagga actcgtatct g 51

<210> SEQ ID NO 24
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
sense oligonucleotide Arg158Gln mutation

<400> SEQUENCE: 24

gatacgagtt cctgaatggt caggttcagt ccactgagtt gtgtg 45

<210> SEQ ID NO 25
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
antisense oligonucleotide Arg158Gln mutation

<400> SEQUENCE: 25

cacacaactc agtggactga acctgaccat tcaggaactc gtatc 45

<210> SEQ ID NO 26
<211> LENGTH: 812
<212> TYPE: PRT
<213> ORGANISM: Canis familiaris

<400> SEQUENCE: 26

```
Met Glu His Lys Glu Val Val Leu Leu Leu Leu Leu Phe Leu Lys Ser
1               5                   10                  15

Gly His Gly Ser Leu Leu Asp Asp Tyr Val Asn Thr Gln Gly Ala Ser
            20                  25                  30

Val Phe Ser Leu Thr Lys Lys Gln Leu Ser Val Gly Ser Ile Glu Glu
        35                  40                  45

Cys Ala Ala Lys Cys Glu Glu Glu Thr Gly Phe Ile Cys Arg Ser Phe
    50                  55                  60

Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Pro Glu Asn Ser
65                  70                  75                  80

Lys Ser Ser Ile Val Phe Arg Met Arg Asp Val Phe Leu Phe Glu Lys
                85                  90                  95

Arg Ile Tyr Leu Ser Glu Cys Lys Thr Gly Asn Gly Lys Thr Tyr Arg
            100                 105                 110

Gly Thr Met Ala Lys Thr Lys Asn Asp Val Ala Cys Gln Lys Trp Ser
        115                 120                 125

Asp Asn Ser Pro His Lys Pro Asn Tyr Thr Pro Glu Lys His Pro Leu
    130                 135                 140

Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Asn
145                 150                 155                 160

Gly Pro Trp Cys Tyr Thr Thr Asn Pro Asp Val Arg Phe Asp Tyr Cys
                165                 170                 175

Asn Ile Pro Glu Cys Glu Glu Glu Cys Met His Cys Ser Gly Glu Asn
            180                 185                 190

Tyr Glu Gly Lys Ile Ser Lys Thr Lys Ser Gly Leu Glu Cys Gln Ala
        195                 200                 205

Trp Asn Ser Gln Thr Pro His Ala His Gly Tyr Ile Pro Ser Lys Phe
    210                 215                 220

Pro Ser Lys Asn Leu Lys Met Asn Tyr Cys Arg Asn Pro Asp Gly Glu
225                 230                 235                 240

Pro Arg Pro Trp Cys Phe Thr Met Asp Pro Asn Lys Arg Trp Glu Phe
                245                 250                 255

Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Ser Gly Pro Thr
            260                 265                 270

Tyr Gln Cys Leu Lys Gly Arg Gly Glu Ser Tyr Arg Gly Lys Val Ser
        275                 280                 285

Val Thr Val Ser Gly His Thr Cys Gln His Trp Ser Glu Gln Thr Pro
    290                 295                 300

His Lys His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Asp
305                 310                 315                 320

Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr
                325                 330                 335

Thr Thr Asn Ser Glu Val Arg Trp Glu His Cys Gln Ile Pro Ser Cys
            340                 345                 350

Glu Ser Ser Pro Ile Thr Thr Glu Tyr Leu Asp Ala Pro Ala Ser Val
        355                 360                 365

Pro Pro Glu Gln Thr Pro Val Val Gln Glu Cys Tyr His Gly Asn Gly
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    370                 375                 380

Gln Ser Tyr Arg Gly Thr Ser Ser Thr Thr Ile Thr Gly Arg Lys Cys
385                 390                 395                 400

Gln Ser Trp Ser Ser Met Thr Pro His Arg His Glu Lys Thr Pro Glu
                405                 410                 415

His Phe Pro Glu Ala Gly Leu Thr Met Asn Tyr Cys Arg Asn Pro Asp
            420                 425                 430

Ala Asp Lys Ser Pro Trp Cys Tyr Thr Thr Asp Pro Ser Val Arg Trp
        435                 440                 445

Glu Phe Cys Asn Leu Arg Lys Cys Leu Asp Pro Glu Ala Ser Ala Thr
    450                 455                 460

Asn Ser Pro Ala Val Pro Gln Val Pro Ser Gly Gln Glu Pro Ser Ala
465                 470                 475                 480

Ser Asp Cys Met Phe Gly Asn Gly Lys Gly Tyr Arg Gly Lys Lys Ala
                485                 490                 495

Thr Thr Val Met Gly Ile Pro Cys Gln Glu Trp Ala Ala Gln Glu Pro
            500                 505                 510

His Arg His Ser Ile Phe Thr Pro Glu Thr Asn Pro Gln Ala Gly Leu
        515                 520                 525

Glu Lys Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Asn Gly Pro Trp
    530                 535                 540

Cys Tyr Thr Met Asn Gln Arg Lys Leu Phe Asp Tyr Cys Asp Val Pro
545                 550                 555                 560

Gln Cys Val Ser Thr Ser Phe Asp Cys Gly Lys Pro Gln Val Glu Pro
                565                 570                 575

Lys Lys Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala Asn Pro His
            580                 585                 590

Ser Trp Pro Trp Gln Ile Ser Leu Arg Thr Arg Tyr Gly Lys His Phe
        595                 600                 605

Cys Gly Gly Thr Leu Ile Ser Pro Glu Trp Val Leu Thr Ala Ala His
    610                 615                 620

Cys Leu Glu Arg Ser Ser Arg Pro Ala Ser Tyr Lys Val Ile Leu Gly
625                 630                 635                 640

Ala His Lys Glu Val Asn Leu Glu Ser Asp Val Gln Glu Ile Glu Val
                645                 650                 655

Tyr Lys Leu Phe Leu Glu Pro Thr Arg Ala Asp Ile Ala Leu Leu Lys
            660                 665                 670

Leu Ser Ser Pro Ala Val Ile Thr Ser Lys Val Ile Pro Ala Cys Leu
        675                 680                 685

Pro Pro Pro Asn Tyr Val Val Ala Asp Arg Thr Leu Cys Tyr Ile Thr
    690                 695                 700

Gly Trp Gly Glu Thr Gln Gly Thr Tyr Gly Ala Gly Leu Leu Lys Glu
705                 710                 715                 720

Ala Gln Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Tyr Glu Tyr
                725                 730                 735

Leu Asn Gly Arg Val Lys Ser Thr Glu Leu Cys Ala Gly Asn Leu Ala
            740                 745                 750

Gly Gly Thr Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys
        755                 760                 765

Phe Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu
    770                 775                 780

Gly Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg
785                 790                 795                 800
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Phe Val Thr Trp Ile Glu Gly Ile Met Arg Asn Asn
                805                 810


<210> SEQ ID NO 27
<211> LENGTH: 810
<212> TYPE: PRT
<213> ORGANISM: Pan troglodytes

<400> SEQUENCE: 27

Met Glu His Lys Glu Val Val Leu Leu Leu Leu Leu Phe Leu Lys Ser
1               5                   10                  15

Gly Gln Gly Glu Pro Leu Asp Asp Tyr Val Asn Thr Gln Gly Ala Ser
            20                  25                  30

Leu Phe Ser Val Thr Lys Lys Gln Leu Gly Ala Gly Ser Ile Glu Glu
        35                  40                  45

Cys Ala Ala Lys Cys Glu Glu Asp Lys Glu Phe Thr Cys Arg Ala Phe
    50                  55                  60

Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Arg
65                  70                  75                  80

Lys Ser Ser Ile Ile Ile Arg Met Arg Asp Val Val Leu Phe Glu Lys
                85                  90                  95

Lys Val Tyr Leu Ser Glu Cys Lys Thr Gly Asn Gly Lys Asn Tyr Arg
            100                 105                 110

Gly Thr Met Ser Lys Thr Lys Asn Gly Ile Thr Cys Gln Lys Trp Ser
        115                 120                 125

Ser Thr Ser Pro His Arg Pro Arg Phe Ser Pro Ala Thr His Pro Ser
    130                 135                 140

Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Pro Gln
145                 150                 155                 160

Gly Pro Trp Cys Tyr Thr Thr Asp Pro Glu Lys Arg Tyr Asp Tyr Cys
                165                 170                 175

Asp Ile Leu Glu Cys Glu Glu Glu Cys Met His Cys Ser Gly Glu Asn
            180                 185                 190

Tyr Asp Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Glu Cys Gln Ala
        195                 200                 205

Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ser Lys Phe
    210                 215                 220

Pro Asn Lys Asn Leu Lys Lys Asn Tyr Cys Arg Asn Pro Asp Gly Glu
225                 230                 235                 240

Leu Arg Pro Trp Cys Phe Thr Thr Asp Pro Asn Lys Arg Trp Glu Leu
                245                 250                 255

Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Ser Ser Gly Pro Thr
            260                 265                 270

Tyr Gln Cys Leu Lys Gly Thr Gly Glu Asn Tyr Arg Gly Asn Val Ala
        275                 280                 285

Val Thr Val Ser Gly His Thr Cys Gln His Trp Ser Ala Gln Thr Pro
    290                 295                 300

His Thr His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Asp
305                 310                 315                 320

Glu Asn Tyr Cys Arg Asn Pro Asp Gly Lys Arg Ala Pro Trp Cys His
                325                 330                 335

Thr Thr Asn Ser Gln Val Arg Trp Glu Tyr Cys Lys Ile Pro Ser Cys
            340                 345                 350

Asp Ser Ser Leu Val Ser Thr Glu Gln Leu Ala Pro Thr Ala Pro Pro
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        355                 360                 365

Glu Leu Thr Pro Val Val Gln Asp Cys Tyr His Gly Asp Gly Gln Ser
    370                 375                 380

Tyr Arg Gly Thr Ser Ser Thr Thr Thr Thr Gly Lys Lys Cys Gln Ser
385                 390                 395                 400

Trp Ser Ser Met Thr Pro His Arg His Gln Lys Thr Pro Glu Asn Tyr
                405                 410                 415

Pro Asn Ala Gly Leu Thr Met Asn Tyr Cys Arg Asn Pro Asp Ala Asp
            420                 425                 430

Lys Gly Pro Trp Cys Phe Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr
        435                 440                 445

Cys Asn Leu Lys Lys Cys Ser Gly Thr Glu Ala Ser Val Val Ala Pro
    450                 455                 460

Pro Pro Val Val Gln Leu Pro Asn Val Glu Thr Pro Ser Glu Glu Asp
465                 470                 475                 480

Cys Met Phe Gly Asn Gly Lys Gly Tyr Arg Gly Lys Arg Ala Thr Thr
                485                 490                 495

Val Thr Gly Thr Pro Cys Gln Asp Trp Ala Ala Gln Glu Pro His Arg
            500                 505                 510

His Ser Ile Phe Thr Pro Glu Thr Asn Pro Arg Ala Gly Leu Glu Lys
        515                 520                 525

Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Gly Gly Pro Trp Cys Tyr
    530                 535                 540

Thr Thr Asn Pro Arg Lys Leu Tyr Asp Tyr Cys Asp Val Pro Gln Cys
545                 550                 555                 560

Ala Ser Pro Ser Phe Asp Cys Gly Lys Pro Gln Val Glu Pro Lys Lys
                565                 570                 575

Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala His Pro His Ser Trp
            580                 585                 590

Pro Trp Gln Val Ser Leu Arg Thr Arg Leu Gly Met His Phe Cys Gly
        595                 600                 605

Gly Thr Leu Ile Ser Pro Glu Trp Val Leu Thr Ala Ala His Cys Leu
    610                 615                 620

Glu Lys Ser Pro Arg Pro Ser Ser Tyr Lys Val Ile Leu Gly Ala His
625                 630                 635                 640

Gln Glu Val Lys Leu Glu Pro His Val Gln Glu Ile Glu Val Ser Arg
                645                 650                 655

Leu Phe Leu Glu Pro Thr Arg Thr Asp Ile Ala Leu Leu Lys Leu Ser
            660                 665                 670

Ser Pro Ala Ile Ile Thr Asp Lys Val Ile Pro Ala Cys Leu Pro Ser
        675                 680                 685

Pro Asn Tyr Val Val Ala Asp Arg Thr Glu Cys Phe Ile Thr Gly Trp
    690                 695                 700

Gly Glu Thr Gln Gly Thr Phe Gly Ala Gly Leu Leu Lys Glu Ala Gln
705                 710                 715                 720

Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Asn Glu Phe Leu Asn
                725                 730                 735

Gly Arg Val Lys Ser Thr Glu Leu Cys Ala Gly His Leu Ala Gly Gly
            740                 745                 750

Thr Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Phe Glu
        755                 760                 765

Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu Gly Cys
    770                 775                 780
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Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg Phe Val
785                 790                 795                 800

Thr Trp Ile Glu Gly Val Met Arg Asn Asn
                805                 810


<210> SEQ ID NO 28
<211> LENGTH: 815
<212> TYPE: PRT
<213> ORGANISM: Pan troglodytes

<400> SEQUENCE: 28

Met Glu His Lys Glu Val Val Leu Leu Leu Leu Leu Phe Leu Lys Ser
1               5                   10                  15

Gly Gln Gly Glu Pro Leu Asp Asp Tyr Val Asn Thr Gln Gly Ala Ser
            20                  25                  30

Leu Phe Ser Val Thr Lys Lys Gln Leu Gly Ala Gly Ser Ile Glu Glu
        35                  40                  45

Cys Ala Ala Lys Cys Glu Glu Asp Lys Glu Phe Thr Cys Arg Ala Phe
    50                  55                  60

Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Arg
65                  70                  75                  80

Lys Ser Ser Ile Ile Ile Arg Met Arg Asp Val Val Leu Phe Glu Lys
                85                  90                  95

Lys Val Tyr Leu Ser Glu Cys Lys Thr Gly Asn Gly Lys Asn Tyr Arg
            100                 105                 110

Gly Thr Met Ser Lys Thr Lys Asn Gly Ile Thr Cys Gln Lys Trp Ser
        115                 120                 125

Ser Thr Ser Pro His Arg Pro Arg Phe Ser Pro Ala Thr His Pro Ser
    130                 135                 140

Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Pro Gln
145                 150                 155                 160

Gly Pro Trp Cys Tyr Thr Thr Asp Pro Glu Lys Arg Tyr Asp Tyr Cys
                165                 170                 175

Asp Ile Leu Glu Cys Glu Glu Glu Cys Met His Cys Ser Gly Glu Asn
            180                 185                 190

Tyr Asp Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Glu Cys Gln Ala
        195                 200                 205

Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ser Lys Phe
    210                 215                 220

Pro Asn Lys Asn Leu Lys Lys Asn Tyr Cys Arg Asn Pro Asp Gly Glu
225                 230                 235                 240

Leu Arg Pro Trp Cys Phe Thr Thr Asp Pro Asn Lys Arg Trp Glu Leu
                245                 250                 255

Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Ser Ser Gly Pro Thr
            260                 265                 270

Tyr Gln Cys Leu Lys Gly Thr Gly Glu Asn Tyr Arg Gly Asn Val Ala
        275                 280                 285

Val Thr Val Ser Gly His Thr Cys Gln His Trp Ser Ala Gln Thr Pro
    290                 295                 300

His Thr His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Asp
305                 310                 315                 320

Glu Asn Tyr Cys Arg Asn Pro Asp Gly Lys Arg Ala Pro Trp Cys His
                325                 330                 335

Thr Thr Asn Ser Gln Val Arg Trp Glu Tyr Cys Lys Ile Pro Ser Cys
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            340                 345                 350

Asp Ser Ser Leu Val Ser Thr Glu Gln Leu Ala Pro Thr Ala Pro Pro
        355                 360                 365

Glu Leu Thr Pro Val Val Gln Asp Cys Tyr His Gly Asp Gly Gln Ser
    370                 375                 380

Tyr Arg Gly Thr Ser Ser Thr Thr Thr Thr Gly Lys Lys Cys Gln Ser
385                 390                 395                 400

Trp Ser Ser Met Thr Pro His Arg His Gln Lys Thr Pro Glu Asn Tyr
                405                 410                 415

Pro Asn Ala Gly Leu Thr Met Asn Tyr Cys Arg Asn Pro Asp Ala Asp
            420                 425                 430

Lys Gly Pro Trp Cys Phe Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr
        435                 440                 445

Cys Asn Leu Lys Lys Cys Ser Gly Thr Glu Ala Ser Val Val Ala Pro
    450                 455                 460

Pro Pro Val Val Gln Leu Pro Asn Val Glu Thr Pro Ser Glu Glu Asp
465                 470                 475                 480

Cys Met Phe Gly Asn Gly Lys Gly Tyr Arg Gly Lys Arg Ala Thr Thr
                485                 490                 495

Val Thr Gly Thr Pro Cys Gln Asp Trp Ala Ala Gln Glu Pro His Arg
            500                 505                 510

His Ser Ile Phe Thr Pro Glu Thr Asn Pro Arg Ala Gly Leu Glu Lys
        515                 520                 525

Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Gly Gly Pro Trp Cys Tyr
    530                 535                 540

Thr Thr Asn Pro Arg Lys Leu Tyr Asp Tyr Cys Asp Val Pro Gln Cys
545                 550                 555                 560

Ala Ser Pro Ser Phe Asp Cys Gly Lys Pro Gln Val Glu Pro Lys Lys
                565                 570                 575

Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala His Pro His Ser Trp
            580                 585                 590

Pro Trp Gln Val Ser Leu Arg Thr Ser Ser Asn Ile Ala Gly Lys Tyr
        595                 600                 605

Trp His Phe Cys Gly Gly Thr Leu Ile Ser Pro Glu Trp Val Leu Thr
    610                 615                 620

Ala Ala His Cys Leu Glu Lys Ser Pro Arg Pro Ser Ser Tyr Lys Val
625                 630                 635                 640

Ile Leu Gly Ala His Gln Glu Val Lys Leu Glu Pro His Val Gln Glu
                645                 650                 655

Ile Glu Val Ser Arg Leu Phe Leu Glu Pro Thr Arg Thr Asp Ile Ala
            660                 665                 670

Leu Leu Lys Leu Ser Ser Pro Ala Ile Ile Thr Asp Lys Val Ile Pro
        675                 680                 685

Ala Cys Leu Pro Ser Pro Asn Tyr Val Val Ala Asp Arg Thr Glu Cys
    690                 695                 700

Phe Ile Thr Gly Trp Gly Glu Thr Gln Gly Thr Phe Gly Ala Gly Leu
705                 710                 715                 720

Leu Lys Glu Ala Gln Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg
                725                 730                 735

Asn Glu Phe Leu Asn Gly Arg Val Lys Ser Thr Glu Leu Cys Ala Gly
            740                 745                 750

His Leu Ala Gly Gly Thr Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro
        755                 760                 765
```

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Leu Val Cys Phe Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser
    770                 775                 780

Trp Gly Leu Gly Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg
785                 790                 795                 800

Val Ser Arg Phe Val Thr Trp Ile Glu Gly Val Met Arg Asn Asn
                805                 810                 815


<210> SEQ ID NO 29
<211> LENGTH: 800
<212> TYPE: PRT
<213> ORGANISM: Pan troglodytes

<400> SEQUENCE: 29

Met Leu Met Asp Tyr Glu Gly Gln Gly Glu Pro Leu Asp Asp Tyr Val
1               5                   10                  15

Asn Thr Gln Gly Ala Ser Leu Phe Ser Val Thr Lys Lys Gln Leu Gly
            20                  25                  30

Ala Gly Ser Ile Glu Glu Cys Ala Ala Lys Cys Glu Glu Asp Lys Glu
        35                  40                  45

Phe Thr Cys Arg Ala Phe Gln Tyr His Ser Lys Glu Gln Gln Cys Val
    50                  55                  60

Ile Met Ala Glu Asn Arg Lys Ser Ser Ile Ile Ile Arg Met Arg Asp
65                  70                  75                  80

Val Val Leu Phe Glu Lys Lys Val Tyr Leu Ser Glu Cys Lys Thr Gly
                85                  90                  95

Asn Gly Lys Asn Tyr Arg Gly Thr Met Ser Lys Thr Lys Asn Gly Ile
            100                 105                 110

Thr Cys Gln Lys Trp Ser Ser Thr Ser Pro His Arg Pro Arg Phe Ser
        115                 120                 125

Pro Ala Thr His Pro Ser Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn
    130                 135                 140

Pro Asp Asn Asp Pro Gln Gly Pro Trp Cys Tyr Thr Thr Asp Pro Glu
145                 150                 155                 160

Lys Arg Tyr Asp Tyr Cys Asp Ile Leu Glu Cys Glu Glu Glu Cys Met
                165                 170                 175

His Cys Ser Gly Glu Asn Tyr Asp Gly Lys Ile Ser Lys Thr Met Ser
            180                 185                 190

Gly Leu Glu Cys Gln Ala Trp Asp Ser Gln Ser Pro His Ala His Gly
        195                 200                 205

Tyr Ile Pro Ser Lys Phe Pro Asn Lys Asn Leu Lys Lys Asn Tyr Cys
    210                 215                 220

Arg Asn Pro Asp Gly Glu Leu Arg Pro Trp Cys Phe Thr Thr Asp Pro
225                 230                 235                 240

Asn Lys Arg Trp Glu Leu Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro
                245                 250                 255

Pro Ser Ser Gly Pro Thr Tyr Gln Cys Leu Lys Gly Thr Gly Glu Asn
            260                 265                 270

Tyr Arg Gly Asn Val Ala Val Thr Val Ser Gly His Thr Cys Gln His
        275                 280                 285

Trp Ser Ala Gln Thr Pro His Thr His Asn Arg Thr Pro Glu Asn Phe
    290                 295                 300

Pro Cys Lys Asn Leu Asp Glu Asn Tyr Cys Arg Asn Pro Asp Gly Lys
305                 310                 315                 320

Arg Ala Pro Trp Cys His Thr Thr Asn Ser Gln Val Arg Trp Glu Tyr
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                325                 330                 335

Cys Lys Ile Pro Ser Cys Asp Ser Ser Leu Val Ser Thr Glu Gln Leu
            340                 345                 350

Ala Pro Thr Ala Pro Pro Glu Leu Thr Pro Val Val Gln Asp Cys Tyr
        355                 360                 365

His Gly Asp Gly Gln Ser Tyr Arg Gly Thr Ser Ser Thr Thr Thr Thr
    370                 375                 380

Gly Lys Lys Cys Gln Ser Trp Ser Ser Met Thr Pro His Arg His Gln
385                 390                 395                 400

Lys Thr Pro Glu Asn Tyr Pro Asn Ala Gly Leu Thr Met Asn Tyr Cys
                405                 410                 415

Arg Asn Pro Asp Ala Asp Lys Gly Pro Trp Cys Phe Thr Thr Asp Pro
            420                 425                 430

Ser Val Arg Trp Glu Tyr Cys Asn Leu Lys Lys Cys Ser Gly Thr Glu
        435                 440                 445

Ala Ser Val Val Ala Pro Pro Pro Val Val Gln Leu Pro Asn Val Glu
    450                 455                 460

Thr Pro Ser Glu Glu Asp Cys Met Phe Gly Asn Gly Lys Gly Tyr Arg
465                 470                 475                 480

Gly Lys Arg Ala Thr Thr Val Thr Gly Thr Pro Cys Gln Asp Trp Ala
                485                 490                 495

Ala Gln Glu Pro His Arg His Ser Ile Phe Thr Pro Glu Thr Asn Pro
            500                 505                 510

Arg Ala Gly Leu Glu Lys Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val
        515                 520                 525

Gly Gly Pro Trp Cys Tyr Thr Thr Asn Pro Arg Lys Leu Tyr Asp Tyr
    530                 535                 540

Cys Asp Val Pro Gln Cys Ala Ser Pro Ser Phe Asp Cys Gly Lys Pro
545                 550                 555                 560

Gln Val Glu Pro Lys Lys Cys Pro Gly Arg Val Val Gly Gly Cys Val
                565                 570                 575

Ala His Pro His Ser Trp Pro Trp Gln Val Ser Leu Arg Thr Arg Leu
            580                 585                 590

Gly Met His Phe Cys Gly Gly Thr Leu Ile Ser Pro Glu Trp Val Leu
        595                 600                 605

Thr Ala Ala His Cys Leu Glu Lys Ser Pro Arg Pro Ser Ser Tyr Lys
    610                 615                 620

Val Ile Leu Gly Ala His Gln Glu Val Lys Leu Glu Pro His Val Gln
625                 630                 635                 640

Glu Ile Glu Val Ser Arg Leu Phe Leu Glu Pro Thr Arg Thr Asp Ile
                645                 650                 655

Ala Leu Leu Lys Leu Ser Ser Pro Ala Ile Ile Thr Asp Lys Val Ile
            660                 665                 670

Pro Ala Cys Leu Pro Ser Pro Asn Tyr Val Val Ala Asp Arg Thr Glu
        675                 680                 685

Cys Phe Ile Thr Gly Trp Gly Glu Thr Gln Gly Thr Phe Gly Ala Gly
    690                 695                 700

Leu Leu Lys Glu Ala Gln Leu Pro Val Ile Glu Asn Lys Val Cys Asn
705                 710                 715                 720

Arg Asn Glu Phe Leu Asn Gly Arg Val Lys Ser Thr Glu Leu Cys Ala
                725                 730                 735

Gly His Leu Ala Gly Gly Thr Asp Ser Cys Gln Gly Asp Ser Gly Gly
            740                 745                 750
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Pro Leu Val Cys Phe Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr
        755                 760                 765

Ser Trp Gly Leu Gly Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val
    770                 775                 780

Arg Val Ser Arg Phe Val Thr Trp Ile Glu Gly Val Met Arg Asn Asn
785                 790                 795                 800


<210> SEQ ID NO 30
<211> LENGTH: 810
<212> TYPE: PRT
<213> ORGANISM: Macaca mulatta

<400> SEQUENCE: 30

Met Glu His Lys Glu Val Val Leu Leu Leu Leu Leu Phe Leu Lys Ser
1               5                   10                  15

Gly Gln Gly Glu Pro Leu Asp Asp Tyr Val Asn Thr Lys Gly Ala Ser
            20                  25                  30

Leu Phe Ser Ile Thr Lys Lys Gln Leu Gly Ala Gly Ser Ile Glu Glu
        35                  40                  45

Cys Ala Ala Lys Cys Glu Glu Glu Glu Glu Phe Thr Cys Arg Ser Phe
    50                  55                  60

Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Arg
65                  70                  75                  80

Lys Ser Ser Ile Val Phe Arg Met Arg Asp Val Val Leu Phe Glu Lys
                85                  90                  95

Lys Val Tyr Leu Ser Glu Cys Lys Thr Gly Asn Gly Lys Asn Tyr Arg
            100                 105                 110

Gly Thr Met Ser Lys Thr Arg Thr Gly Ile Thr Cys Gln Lys Trp Ser
        115                 120                 125

Ser Thr Ser Pro His Arg Pro Thr Phe Ser Pro Ala Thr His Pro Ser
    130                 135                 140

Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Gly Gln
145                 150                 155                 160

Gly Pro Trp Cys Tyr Thr Thr Asp Pro Glu Glu Arg Phe Asp Tyr Cys
                165                 170                 175

Asp Ile Pro Glu Cys Glu Asp Glu Cys Met His Cys Ser Gly Glu Asn
            180                 185                 190

Tyr Asp Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Glu Cys Gln Ala
        195                 200                 205

Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ser Lys Phe
    210                 215                 220

Pro Asn Lys Asn Leu Lys Lys Asn Tyr Cys Arg Asn Pro Asp Gly Glu
225                 230                 235                 240

Pro Arg Pro Trp Cys Phe Thr Thr Asp Pro Asn Lys Arg Trp Glu Leu
                245                 250                 255

Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Ser Ser Gly Pro Thr
            260                 265                 270

Tyr Gln Cys Leu Lys Gly Thr Gly Glu Asn Tyr Arg Gly Asp Val Ala
        275                 280                 285

Val Thr Val Ser Gly His Thr Cys His Gly Trp Ser Ala Gln Thr Pro
    290                 295                 300

His Thr His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Asp
305                 310                 315                 320

Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Lys Ala Pro Trp Cys Tyr
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                325                 330                 335

Thr Thr Asn Ser Gln Val Arg Trp Glu Tyr Cys Lys Ile Pro Ser Cys
            340                 345                 350

Glu Ser Ser Pro Val Ser Thr Glu Pro Leu Asp Pro Thr Ala Pro Pro
        355                 360                 365

Glu Leu Thr Pro Val Val Gln Glu Cys Tyr His Gly Asp Gly Gln Ser
    370                 375                 380

Tyr Arg Gly Thr Ser Ser Thr Thr Thr Thr Gly Lys Lys Cys Gln Ser
385                 390                 395                 400

Trp Ser Ser Met Thr Pro His Trp His Glu Lys Thr Pro Glu Asn Phe
                405                 410                 415

Pro Asn Ala Gly Leu Thr Met Asn Tyr Cys Arg Asn Pro Asp Ala Asp
            420                 425                 430

Lys Gly Pro Trp Cys Phe Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr
        435                 440                 445

Cys Asn Leu Lys Lys Cys Ser Gly Thr Glu Gly Ser Val Ala Ala Pro
    450                 455                 460

Pro Pro Val Ala Gln Leu Pro Asp Ala Glu Thr Pro Ser Glu Glu Asp
465                 470                 475                 480

Cys Met Phe Gly Asn Gly Lys Gly Tyr Arg Gly Lys Lys Ala Thr Thr
                485                 490                 495

Val Thr Gly Thr Pro Cys Gln Glu Trp Ala Ala Gln Glu Pro His Ser
            500                 505                 510

His Arg Ile Phe Thr Pro Glu Thr Asn Pro Arg Ala Gly Leu Glu Lys
        515                 520                 525

Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Gly Gly Pro Trp Cys Tyr
    530                 535                 540

Thr Thr Asn Pro Arg Lys Leu Phe Asp Tyr Cys Asp Val Pro Gln Cys
545                 550                 555                 560

Ala Ala Ser Ser Phe Asp Cys Gly Lys Pro Gln Val Glu Pro Lys Lys
                565                 570                 575

Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala Tyr Pro His Ser Trp
            580                 585                 590

Pro Trp Gln Ile Ser Leu Arg Thr Arg Leu Gly Met His Phe Cys Gly
        595                 600                 605

Gly Thr Leu Ile Ser Pro Glu Trp Val Leu Thr Ala Ala His Cys Leu
    610                 615                 620

Glu Lys Ser Ser Arg Pro Ser Phe Tyr Lys Val Ile Leu Gly Ala His
625                 630                 635                 640

Arg Glu Val His Leu Glu Pro His Val Gln Glu Ile Glu Val Ser Lys
                645                 650                 655

Met Phe Ser Glu Pro Ala Arg Ala Asp Ile Ala Leu Leu Lys Leu Ser
            660                 665                 670

Ser Pro Ala Ile Ile Thr Asp Lys Val Ile Pro Ala Cys Leu Pro Ser
        675                 680                 685

Pro Asn Tyr Val Val Ala Asp Arg Thr Glu Cys Phe Ile Thr Gly Trp
    690                 695                 700

Gly Glu Thr Gln Gly Thr Tyr Gly Ala Gly Leu Leu Lys Glu Ala Arg
705                 710                 715                 720

Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Tyr Glu Phe Leu Asn
                725                 730                 735

Gly Thr Val Lys Thr Thr Glu Leu Cys Ala Gly His Leu Ala Gly Gly
            740                 745                 750
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```
Thr Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Phe Glu
        755                 760                 765

Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu Gly Cys
    770                 775                 780

Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg Phe Val
785                 790                 795                 800

Thr Trp Ile Glu Gly Val Met Arg Asn Asn
                805                 810
```

<210> SEQ ID NO 31
<211> LENGTH: 810
<212> TYPE: PRT
<213> ORGANISM: Pongo abelii

<400> SEQUENCE: 31

```
Met Glu His Lys Glu Val Val Leu Leu Leu Leu Leu Phe Leu Lys Ser
1               5                   10                  15

Gly Gln Gly Glu Pro Leu Asp Asp Tyr Val Asn Thr Gln Gly Ala Ser
            20                  25                  30

Leu Phe Ser Val Thr Lys Lys Gln Leu Arg Ala Gly Ser Ile Glu Glu
        35                  40                  45

Cys Ala Ala Lys Cys Glu Glu Glu Lys Glu Phe Thr Cys Arg Ala Phe
    50                  55                  60

Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Arg
65                  70                  75                  80

Lys Ser Ser Ile Ile Ile Arg Met Arg Asp Val Val Leu Phe Glu Lys
                85                  90                  95

Lys Val Tyr Leu Ser Glu Cys Lys Thr Gly Asn Gly Lys Asn Tyr Arg
            100                 105                 110

Gly Thr Met Ser Lys Thr Lys Asn Gly Ile Thr Cys Gln Lys Trp Ser
        115                 120                 125

Ser Thr Ser Pro His Arg Pro Arg Phe Ser Pro Ala Thr His Pro Ser
    130                 135                 140

Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Ala Gln
145                 150                 155                 160

Gly Pro Trp Cys Tyr Thr Thr Asp Pro Glu His Arg Tyr Asp Tyr Cys
                165                 170                 175

Asp Ile Pro Glu Cys Glu Glu Ala Cys Met His Cys Ser Gly Glu Asn
            180                 185                 190

Tyr Asp Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Glu Cys Gln Ala
        195                 200                 205

Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ser Lys Phe
    210                 215                 220

Pro Asn Lys Asn Leu Lys Lys Asn Tyr Cys Arg Asn Pro Asp Gly Glu
225                 230                 235                 240

Pro Arg Pro Trp Cys Phe Thr Thr Asp Pro Asn Lys Arg Trp Glu Leu
                245                 250                 255

Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Ser Ser Gly Pro Thr
            260                 265                 270

Tyr Gln Cys Leu Lys Gly Thr Gly Glu Asn Tyr Arg Gly Asn Val Ala
        275                 280                 285

Val Thr Val Ser Gly His Thr Cys Gln Arg Trp Ser Ala Gln Thr Pro
    290                 295                 300

Gln Thr His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Asp
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305                 310                 315                 320

Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Lys Ala Pro Trp Cys Tyr
                325                 330                 335

Thr Thr Asn Ser Gln Val Arg Trp Glu Tyr Cys Lys Ile Pro Ser Cys
            340                 345                 350

Gly Ser Ser Pro Val Ser Thr Glu Gln Leu Asp Pro Thr Ala Pro Pro
        355                 360                 365

Glu Leu Thr Pro Val Val Gln Asp Cys Tyr His Gly Asp Gly Gln Ser
    370                 375                 380

Tyr Arg Gly Thr Ser Ser Thr Thr Thr Thr Gly Lys Lys Cys Gln Ser
385                 390                 395                 400

Trp Ser Ser Met Thr Pro His Trp His Gln Lys Thr Pro Glu Asn Tyr
                405                 410                 415

Pro Asp Ala Gly Leu Thr Met Asn Tyr Cys Arg Asn Pro Asp Ala Asp
            420                 425                 430

Lys Gly Pro Trp Cys Phe Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr
        435                 440                 445

Cys Asn Leu Lys Lys Cys Ser Gly Thr Glu Gly Ser Val Val Ala Pro
    450                 455                 460

Pro Pro Val Val Gln Leu Pro Asn Val Glu Thr Pro Ser Glu Glu Asp
465                 470                 475                 480

Cys Met Phe Gly Asn Gly Lys Gly Tyr Arg Gly Lys Arg Ala Thr Thr
                485                 490                 495

Val Thr Gly Thr Pro Cys Gln Glu Trp Ala Ala Gln Glu Pro His Arg
            500                 505                 510

His Ser Ile Phe Thr Pro Gln Thr Asn Pro Arg Ala Gly Leu Glu Lys
        515                 520                 525

Asn Tyr Cys Arg Asn Pro Asp Gly Asp Glu Gly Gly Pro Trp Cys Tyr
    530                 535                 540

Thr Thr Asn Pro Arg Lys His Tyr Asp Tyr Cys Asp Val Pro Gln Cys
545                 550                 555                 560

Ala Ser Ser Ser Phe Asp Cys Gly Lys Pro Gln Val Glu Pro Lys Lys
                565                 570                 575

Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala Asn Ala His Ser Trp
            580                 585                 590

Pro Trp Gln Val Ser Leu Arg Thr Arg Phe Gly Thr His Phe Cys Gly
        595                 600                 605

Gly Thr Leu Ile Ser Pro Glu Trp Val Leu Thr Ala Ala His Cys Leu
    610                 615                 620

Glu Lys Ser Pro Arg Pro Ser Ser Tyr Lys Val Ile Leu Gly Ala His
625                 630                 635                 640

Gln Glu Val Asn Leu Glu Pro His Val Gln Glu Ile Glu Val Ser Arg
                645                 650                 655

Leu Phe Leu Glu Pro Thr Arg Ala Asp Ile Ala Leu Leu Lys Leu Ser
            660                 665                 670

Ser Pro Ala Val Ile Thr Asp Lys Val Ile Pro Ala Cys Leu Pro Ser
        675                 680                 685

Pro Asn Tyr Val Val Ala Gly Arg Thr Glu Cys Phe Ile Thr Gly Trp
    690                 695                 700

Gly Glu Thr Gln Gly Thr Phe Gly Ala Gly Leu Leu Lys Glu Ala Gln
705                 710                 715                 720

Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Tyr Glu Phe Leu Asn
                725                 730                 735
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```
Gly Arg Val Lys Ser Thr Glu Leu Cys Ala Gly His Leu Ala Gly Gly
            740                 745                 750

Thr Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Phe Glu
        755                 760                 765

Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu Gly Cys
    770                 775                 780

Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg Phe Val
785                 790                 795                 800

Thr Trp Ile Glu Gly Val Met Arg Asn Asn
                805                 810


<210> SEQ ID NO 32
<211> LENGTH: 809
<212> TYPE: PRT
<213> ORGANISM: Sus scrofa

<400> SEQUENCE: 32

Met Asp His Lys Glu Val Val Leu Leu Leu Leu Leu Phe Leu Lys Ser
1               5                   10                  15

Gly Leu Gly Asp Ser Leu Asp Asp Tyr Val Asn Thr Gln Gly Ala Phe
            20                  25                  30

Leu Phe Ser Leu Ser Arg Lys Gln Val Ala Ala Arg Ser Val Glu Glu
        35                  40                  45

Cys Ala Ala Lys Cys Glu Ala Glu Thr Asn Phe Ile Cys Arg Ala Phe
    50                  55                  60

Gln Tyr His Ser Lys Asp Gln Gln Cys Val Val Met Ala Glu Asn Ser
65                  70                  75                  80

Lys Thr Ser Pro Ile Ala Arg Met Arg Asp Val Val Leu Phe Glu Lys
                85                  90                  95

Arg Ile Tyr Leu Ser Glu Cys Lys Thr Gly Asn Gly Lys Asn Tyr Arg
            100                 105                 110

Gly Thr Thr Ser Lys Thr Lys Ser Gly Val Ile Cys Gln Lys Trp Ser
        115                 120                 125

Val Ser Ser Pro His Ile Pro Lys Tyr Ser Pro Glu Lys Phe Pro Leu
    130                 135                 140

Ala Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Lys
145                 150                 155                 160

Gly Pro Trp Cys Tyr Thr Thr Asp Pro Glu Thr Arg Phe Asp Tyr Cys
                165                 170                 175

Asp Ile Pro Glu Cys Glu Asp Glu Cys Met His Cys Ser Gly Glu His
            180                 185                 190

Tyr Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Ile Glu Cys Gln Ser
        195                 200                 205

Trp Gly Ser Gln Ser Pro His Ala His Gly Tyr Leu Pro Ser Lys Phe
    210                 215                 220

Pro Asn Lys Asn Leu Lys Met Asn Tyr Cys Arg Asn Pro Asp Gly Glu
225                 230                 235                 240

Pro Arg Pro Trp Cys Phe Thr Thr Asp Pro Asn Lys Arg Trp Glu Phe
                245                 250                 255

Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Thr Ser Gly Pro Thr
            260                 265                 270

Tyr Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser
        275                 280                 285

Val Thr Ala Ser Gly His Thr Cys Gln Arg Trp Ser Ala Gln Ser Pro
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    290                 295                 300

His Lys His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu
305                 310                 315                 320

Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr
                325                 330                 335

Thr Thr Asp Ser Glu Val Arg Trp Asp Tyr Cys Lys Ile Pro Ser Cys
            340                 345                 350

Gly Ser Ser Thr Thr Ser Thr Glu Tyr Leu Asp Ala Pro Val Pro Pro
        355                 360                 365

Glu Gln Thr Pro Val Ala Gln Asp Cys Tyr Arg Gly Asn Gly Glu Ser
    370                 375                 380

Tyr Arg Gly Thr Ser Ser Thr Thr Ile Thr Gly Arg Lys Cys Gln Ser
385                 390                 395                 400

Trp Val Ser Met Thr Pro His Arg His Glu Lys Thr Pro Gly Asn Phe
                405                 410                 415

Pro Asn Ala Gly Leu Thr Met Asn Tyr Cys Arg Asn Pro Asp Ala Asp
            420                 425                 430

Lys Ser Pro Trp Cys Tyr Thr Thr Asp Pro Arg Val Arg Trp Glu Tyr
        435                 440                 445

Cys Asn Leu Lys Lys Cys Ser Glu Thr Glu Gln Gln Val Thr Asn Phe
    450                 455                 460

Pro Ala Ile Ala Gln Val Pro Ser Val Glu Asp Leu Ser Glu Asp Cys
465                 470                 475                 480

Met Phe Gly Asn Gly Lys Arg Tyr Arg Gly Lys Arg Ala Thr Thr Val
                485                 490                 495

Ala Gly Val Pro Cys Gln Glu Trp Ala Ala Gln Glu Pro His Arg His
            500                 505                 510

Ser Ile Phe Thr Pro Glu Thr Asn Pro Arg Ala Gly Leu Glu Lys Asn
        515                 520                 525

Tyr Cys Arg Asn Pro Asp Gly Asp Asp Asn Gly Pro Trp Cys Tyr Thr
    530                 535                 540

Thr Asn Pro Gln Lys Leu Phe Asp Tyr Cys Asp Val Pro Gln Cys Val
545                 550                 555                 560

Thr Ser Ser Phe Asp Cys Gly Lys Pro Lys Val Glu Pro Lys Lys Cys
                565                 570                 575

Pro Ala Arg Val Val Gly Gly Cys Val Ser Ile Pro His Ser Trp Pro
            580                 585                 590

Trp Gln Ile Ser Leu Arg His Arg Tyr Gly Gly His Phe Cys Gly Gly
        595                 600                 605

Thr Leu Ile Ser Pro Glu Trp Val Leu Thr Ala Lys His Cys Leu Glu
    610                 615                 620

Lys Ser Ser Ser Pro Ser Ser Tyr Lys Val Ile Leu Gly Ala His Glu
625                 630                 635                 640

Glu Tyr His Leu Gly Glu Gly Val Gln Glu Ile Asp Val Ser Lys Leu
                645                 650                 655

Phe Lys Glu Pro Ser Glu Ala Asp Ile Ala Leu Leu Lys Leu Ser Ser
            660                 665                 670

Pro Ala Ile Ile Thr Asp Lys Val Ile Pro Ala Cys Leu Pro Thr Pro
        675                 680                 685

Asn Tyr Val Val Ala Asp Arg Thr Ala Cys Tyr Ile Thr Gly Trp Gly
    690                 695                 700

Glu Thr Lys Gly Thr Tyr Gly Ala Gly Leu Leu Lys Glu Ala Arg Leu
705                 710                 715                 720
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Pro Val Ile Glu Asn Lys Val Cys Asn Arg Tyr Glu Tyr Leu Gly Gly
                725                 730                 735

Lys Val Ser Pro Asn Glu Leu Cys Ala Gly His Leu Ala Gly Gly Ile
            740                 745                 750

Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Phe Glu Lys
        755                 760                 765

Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu Gly Cys Ala
    770                 775                 780

Leu Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg Phe Val Thr
785                 790                 795                 800

Trp Ile Glu Glu Ile Met Arg Arg Asn
                805


<210> SEQ ID NO 33
<211> LENGTH: 812
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 33

Met Leu Pro Ala Ser Pro Lys Met Glu His Lys Ala Val Val Phe Leu
1               5                   10                  15

Ile Leu Leu Phe Leu Lys Ser Gly Leu Gly Asp Leu Leu Asp Asp Tyr
            20                  25                  30

Val Asn Thr Gln Gly Ala Ser Leu Leu Ser Leu Ser Arg Lys Asn Leu
        35                  40                  45

Ala Gly Arg Ser Val Glu Asp Cys Ala Ala Lys Cys Glu Glu Glu Thr
    50                  55                  60

Asp Phe Val Cys Arg Ala Phe Gln Tyr His Ser Lys Glu Gln Gln Cys
65                  70                  75                  80

Val Val Met Ala Glu Asn Ser Lys Asn Thr Pro Val Phe Arg Met Arg
                85                  90                  95

Asp Val Ile Leu Tyr Glu Lys Arg Ile Tyr Leu Leu Glu Cys Lys Thr
            100                 105                 110

Gly Asn Gly Gln Thr Tyr Arg Gly Thr Thr Ala Glu Thr Lys Ser Gly
        115                 120                 125

Val Thr Cys Gln Lys Trp Ser Ala Thr Ser Pro His Val Pro Lys Phe
    130                 135                 140

Ser Pro Glu Lys Phe Pro Leu Ala Gly Leu Glu Glu Asn Tyr Cys Arg
145                 150                 155                 160

Asn Pro Asp Asn Asp Glu Asn Gly Pro Trp Cys Tyr Thr Thr Asp Pro
                165                 170                 175

Asp Lys Arg Tyr Asp Tyr Cys Asp Ile Pro Glu Cys Glu Asp Lys Cys
            180                 185                 190

Met His Cys Ser Gly Glu Asn Tyr Glu Gly Lys Ile Ala Lys Thr Met
        195                 200                 205

Ser Gly Arg Asp Cys Gln Ala Trp Asp Ser Gln Ser Pro His Ala His
    210                 215                 220

Gly Tyr Ile Pro Ser Lys Phe Pro Ser Lys Asn Leu Lys Met Asn Tyr
225                 230                 235                 240

Cys Arg Asn Pro Asp Gly Glu Pro Arg Pro Trp Cys Phe Thr Thr Asp
                245                 250                 255

Pro Gln Lys Arg Trp Glu Phe Cys Asp Ile Pro Arg Cys Thr Thr Pro
            260                 265                 270

Pro Pro Ser Ser Gly Pro Lys Tyr Gln Cys Leu Lys Gly Thr Gly Lys
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        275                 280                 285

Asn Tyr Gly Gly Thr Val Ala Val Thr Glu Ser Gly His Thr Cys Gln
    290                 295                 300

Arg Trp Ser Glu Gln Thr Pro His Lys His Asn Arg Thr Pro Glu Asn
305                 310                 315                 320

Phe Pro Cys Lys Asn Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asn Gly
                325                 330                 335

Glu Lys Ala Pro Trp Cys Tyr Thr Thr Asn Ser Lys Val Arg Trp Glu
            340                 345                 350

Tyr Cys Thr Ile Pro Ser Cys Glu Ser Ser Pro Leu Ser Thr Glu Arg
        355                 360                 365

Met Asp Val Pro Val Pro Pro Glu Gln Thr Pro Val Pro Gln Asp Cys
    370                 375                 380

Tyr His Gly Asn Gly Gln Ser Tyr Arg Gly Thr Ser Ser Thr Thr Ile
385                 390                 395                 400

Thr Gly Arg Lys Cys Gln Ser Trp Ser Ser Met Thr Pro His Arg His
                405                 410                 415

Leu Lys Thr Pro Glu Asn Tyr Pro Asn Ala Gly Leu Thr Met Asn Tyr
            420                 425                 430

Cys Arg Asn Pro Asp Ala Asp Lys Ser Pro Trp Cys Tyr Thr Thr Asp
        435                 440                 445

Pro Arg Val Arg Trp Glu Phe Cys Asn Leu Lys Lys Cys Ser Glu Thr
    450                 455                 460

Pro Glu Gln Val Pro Ala Ala Pro Gln Ala Pro Gly Val Glu Asn Pro
465                 470                 475                 480

Pro Glu Ala Asp Cys Met Ile Gly Met Gly Lys Ser Tyr Arg Gly Lys
                485                 490                 495

Lys Ala Thr Thr Val Ala Gly Val Pro Cys Gln Glu Trp Ala Ala Gln
            500                 505                 510

Glu Pro His His His Ser Ile Phe Thr Pro Glu Thr Asn Pro Gln Ser
        515                 520                 525

Gly Leu Glu Arg Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Asn Gly
    530                 535                 540

Pro Trp Cys Tyr Thr Met Asn Pro Arg Lys Leu Phe Asp Tyr Cys Asp
545                 550                 555                 560

Val Pro Gln Cys Glu Ser Ser Phe Asp Cys Gly Lys Pro Lys Val Glu
                565                 570                 575

Pro Lys Lys Cys Ser Gly Arg Ile Val Gly Gly Cys Val Ser Lys Pro
            580                 585                 590

His Ser Trp Pro Trp Gln Val Ser Leu Arg Arg Ser Ser Arg His Phe
        595                 600                 605

Cys Gly Gly Thr Leu Ile Ser Pro Lys Trp Val Leu Thr Ala Ala His
    610                 615                 620

Cys Leu Asp Asn Ile Leu Ala Leu Ser Phe Tyr Lys Val Ile Leu Gly
625                 630                 635                 640

Ala His Asn Glu Lys Val Arg Glu Gln Ser Val Gln Glu Ile Pro Val
                645                 650                 655

Ser Arg Leu Phe Arg Glu Pro Ser Gln Ala Asp Ile Ala Leu Leu Lys
            660                 665                 670

Leu Ser Arg Pro Ala Ile Ile Thr Lys Glu Val Ile Pro Ala Cys Leu
        675                 680                 685

Pro Pro Pro Asn Tyr Met Val Ala Ala Arg Thr Glu Cys Tyr Ile Thr
    690                 695                 700
```

```
Gly Trp Gly Glu Thr Gln Gly Thr Phe Gly Glu Gly Leu Leu Lys Glu
705                 710                 715                 720

Ala His Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Asn Glu Tyr
                725                 730                 735

Leu Asp Gly Arg Val Lys Pro Thr Glu Leu Cys Ala Gly His Leu Ile
            740                 745                 750

Gly Gly Thr Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys
        755                 760                 765

Phe Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu
    770                 775                 780

Gly Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Pro
785                 790                 795                 800

Tyr Val Pro Trp Ile Glu Glu Thr Met Arg Arg Asn
                805                 810


<210> SEQ ID NO 34
<211> LENGTH: 811
<212> TYPE: PRT
<213> ORGANISM: Equus caballus

<400> SEQUENCE: 34

Met Glu His Gln Glu Val Val Phe Leu Leu Leu Leu Phe Leu Lys Ser
1               5                   10                  15

Gly His Gly Asp Ile Leu Asp Asp Tyr Val Thr Thr Gln Gly Ala Ser
            20                  25                  30

Leu Phe Thr Phe Thr Arg Lys Pro Leu Ser Ala Ser Ser Ile Glu Glu
        35                  40                  45

Cys Glu Ala Lys Cys Thr Glu Glu Thr Ala Phe Ile Cys Arg Ala Phe
    50                  55                  60

Gln Tyr His Ser Lys Glu Pro Arg Cys Val Leu Leu Ala Glu Asn Arg
65                  70                  75                  80

Lys Ser Ser Pro Val Met Arg Met Arg Asp Val Ile Leu Phe Glu Lys
                85                  90                  95

Arg Ile Tyr Leu Ser Glu Cys Lys Thr Gly Thr Gly Arg Ser Tyr Arg
            100                 105                 110

Gly Thr Thr Ser Lys Thr Lys Asn Gly Val Ser Cys Gln Lys Trp Ser
        115                 120                 125

Asp Thr Ser Pro His Ile Pro Lys Tyr Ser Pro Asp Lys Asn Pro Ser
    130                 135                 140

Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Lys
145                 150                 155                 160

Gly Pro Trp Cys Tyr Thr Thr Asp Pro Gly Thr Arg Phe Asp Tyr Cys
                165                 170                 175

Asp Ile Pro Glu Cys Glu Asp Glu Cys Met His Cys Ser Gly Glu Asn
            180                 185                 190

Tyr Glu Gly Lys Ile Ser Lys Thr Ile Ser Gly Leu Glu Cys Gln Pro
        195                 200                 205

Trp Ala Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ser Lys Phe
    210                 215                 220

Pro Asn Lys Asn Leu Arg Met Asn Tyr Cys Arg Asn Pro Asp Gly Glu
225                 230                 235                 240

Pro Arg Pro Trp Cys Phe Thr Met Asp Pro Asp Lys Arg Trp Glu Phe
                245                 250                 255

Cys Asp Ile Pro Arg Cys Ser Thr Pro Pro Pro Ser Ser Gly Pro Thr
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            260                 265                 270

Tyr Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Arg Val Ser
        275                 280                 285

Val Thr Gln Ser Gly Leu Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro
    290                 295                 300

His Lys His Asn Arg Thr Pro Asp Asn Phe Pro Cys Lys Asn Leu Asp
305                 310                 315                 320

Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr
                325                 330                 335

Thr Thr Ser Ser Glu Thr Arg Trp Glu Tyr Cys Asn Ile Pro Ser Cys
            340                 345                 350

Thr Ser Ser Ser Val Pro Thr Glu Ile Thr Asp Ala Ser Glu Pro Pro
        355                 360                 365

Glu Gln Thr Pro Val Val Gln Asp Cys Tyr Gln Asp Lys Gly Glu Ser
    370                 375                 380

Tyr Arg Gly Thr Ser Ser Ile Thr Val Thr Gly Lys Lys Cys Gln Ser
385                 390                 395                 400

Trp Ser Ser Met Thr Pro His Trp His Gln Lys Thr Pro Glu Lys Tyr
                405                 410                 415

Pro Asn Ala Asp Leu Thr Met Asn Tyr Cys Arg Asn Pro Asp Gly Asp
            420                 425                 430

Lys Gly Pro Trp Cys Tyr Thr Thr Asp Pro Ser Val Arg Trp Glu Phe
        435                 440                 445

Cys Asn Leu Arg Arg Cys Ser Glu Thr Gln Gln Ser Phe Ser Asn Ser
    450                 455                 460

Ser Pro Thr Asp Thr Gln Val Pro Ser Val Gln Glu Pro Ser Glu Pro
465                 470                 475                 480

Asp Cys Met Leu Gly Ile Gly Lys Gly Tyr Gln Gly Lys Lys Ala Thr
                485                 490                 495

Thr Val Thr Gly Thr Arg Cys Gln Ala Trp Ala Ala Gln Glu Pro His
            500                 505                 510

Arg His Ser Ile Phe Thr Pro Glu Ala Asn Pro Trp Ala Asn Leu Glu
        515                 520                 525

Lys Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Asn Gly Pro Trp Cys
    530                 535                 540

Tyr Thr Met Asn Pro Gln Lys Leu Phe Asp Tyr Cys Asp Val Pro Gln
545                 550                 555                 560

Cys Glu Ser Ser Pro Phe Asp Cys Gly Lys Pro Lys Val Glu Pro Lys
                565                 570                 575

Lys Cys Ser Gly Arg Ile Val Gly Gly Cys Val Ala Ile Ala His Ser
            580                 585                 590

Trp Pro Trp Gln Ile Ser Leu Arg Thr Arg Phe Gly Arg His Phe Cys
        595                 600                 605

Gly Gly Thr Leu Ile Ser Pro Glu Trp Val Leu Thr Ala Ala His Cys
    610                 615                 620

Leu Glu Arg Ser Ser Arg Pro Ser Thr Tyr Lys Val Val Leu Gly Thr
625                 630                 635                 640

His His Glu Leu Arg Leu Ala Ala Gly Ala Gln Gln Ile Asp Val Ser
                645                 650                 655

Lys Leu Phe Leu Glu Pro Ser Arg Ala Asp Ile Ala Leu Leu Lys Leu
            660                 665                 670

Ser Ser Pro Ala Ile Ile Thr Gln Asn Val Ile Pro Ala Cys Leu Pro
        675                 680                 685
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Pro Ala Asp Tyr Val Val Ala Asn Trp Ala Glu Cys Phe Val Thr Gly
    690                 695                 700

Trp Gly Glu Thr Gln Asp Ser Ser Asn Ala Gly Val Leu Lys Glu Ala
705                 710                 715                 720

Gln Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Tyr Glu Tyr Leu
                725                 730                 735

Asn Gly Arg Val Lys Ser Thr Glu Leu Cys Ala Gly His Leu Val Gly
            740                 745                 750

Gly Val Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Phe
        755                 760                 765

Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu Gly
    770                 775                 780

Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Ser Phe
785                 790                 795                 800

Ile Asn Trp Ile Glu Arg Ile Met Gln Ser Asn
                805                 810


<210> SEQ ID NO 35
<211> LENGTH: 812
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 35

Met Asp His Lys Glu Val Ile Leu Leu Phe Leu Leu Leu Leu Lys Pro
1               5                   10                  15

Gly Gln Gly Asp Ser Leu Asp Gly Tyr Ile Ser Thr Gln Gly Ala Ser
            20                  25                  30

Leu Phe Ser Leu Thr Lys Lys Gln Leu Ala Ala Gly Gly Val Ala Asp
        35                  40                  45

Cys Leu Ala Lys Cys Glu Gly Glu Thr Asp Phe Val Cys Arg Ser Phe
    50                  55                  60

Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Ser
65                  70                  75                  80

Lys Thr Ser Ser Ile Ile Arg Met Arg Asp Val Ile Leu Phe Glu Lys
                85                  90                  95

Arg Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Asn Ser Tyr Arg
            100                 105                 110

Gly Thr Met Ser Arg Thr Lys Ser Gly Val Ala Cys Gln Lys Trp Gly
        115                 120                 125

Ala Thr Phe Pro His Val Pro Asn Tyr Ser Pro Ser Thr His Pro Asn
    130                 135                 140

Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln
145                 150                 155                 160

Gly Pro Trp Cys Tyr Thr Thr Asp Pro Asp Lys Arg Tyr Asp Tyr Cys
                165                 170                 175

Asn Ile Pro Glu Cys Glu Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys
            180                 185                 190

Tyr Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Asp Cys Gln Ala
        195                 200                 205

Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ala Lys Phe
    210                 215                 220

Pro Ser Lys Asn Leu Lys Met Asn Tyr Cys Arg Asn Pro Asp Gly Glu
225                 230                 235                 240

Pro Arg Pro Trp Cys Phe Thr Thr Asp Pro Thr Lys Arg Trp Glu Tyr
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                245                 250                 255

Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Ser Pro Thr
            260                 265                 270

Tyr Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser
        275                 280                 285

Val Thr Val Ser Gly Lys Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro
    290                 295                 300

His Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu
305                 310                 315                 320

Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr
                325                 330                 335

Thr Thr Asp Ser Gln Leu Arg Trp Glu Tyr Cys Glu Ile Pro Ser Cys
            340                 345                 350

Glu Ser Ser Ala Ser Pro Asp Gln Ser Asp Ser Ser Val Pro Pro Glu
        355                 360                 365

Glu Gln Thr Pro Val Val Gln Glu Cys Tyr Gln Ser Asp Gly Gln Ser
    370                 375                 380

Tyr Arg Gly Thr Ser Ser Thr Thr Ile Thr Gly Lys Lys Cys Gln Ser
385                 390                 395                 400

Trp Ala Ala Met Phe Pro His Arg His Ser Lys Thr Pro Glu Asn Phe
                405                 410                 415

Pro Asp Ala Gly Leu Glu Met Asn Tyr Cys Arg Asn Pro Asp Gly Asp
            420                 425                 430

Lys Gly Pro Trp Cys Tyr Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr
        435                 440                 445

Cys Asn Leu Lys Arg Cys Ser Glu Thr Gly Gly Ser Val Val Glu Leu
    450                 455                 460

Pro Thr Val Ser Gln Glu Pro Ser Gly Pro Ser Asp Ser Glu Thr Asp
465                 470                 475                 480

Cys Met Tyr Gly Asn Gly Lys Asp Tyr Arg Gly Lys Thr Ala Val Thr
                485                 490                 495

Ala Ala Gly Thr Pro Cys Gln Gly Trp Ala Ala Gln Glu Pro His Arg
            500                 505                 510

His Ser Ile Phe Thr Pro Gln Thr Asn Pro Arg Ala Gly Leu Glu Lys
        515                 520                 525

Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Asn Gly Pro Trp Cys Tyr
    530                 535                 540

Thr Thr Asn Pro Arg Lys Leu Tyr Asp Tyr Cys Asp Ile Pro Leu Cys
545                 550                 555                 560

Ala Ser Ala Ser Ser Phe Glu Cys Gly Lys Pro Gln Val Glu Pro Lys
                565                 570                 575

Lys Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala Asn Pro His Ser
            580                 585                 590

Trp Pro Trp Gln Ile Ser Leu Arg Thr Arg Phe Thr Gly Gln His Phe
        595                 600                 605

Cys Gly Gly Thr Leu Ile Ala Pro Glu Trp Val Leu Thr Ala Ala His
    610                 615                 620

Cys Leu Glu Lys Ser Ser Arg Pro Glu Phe Tyr Lys Val Ile Leu Gly
625                 630                 635                 640

Ala His Glu Glu Tyr Ile Arg Gly Ser Asp Val Gln Glu Ile Ser Val
                645                 650                 655

Ala Lys Leu Ile Leu Glu Pro Asn Asn Arg Asp Ile Ala Leu Leu Lys
            660                 665                 670
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Leu Ser Arg Pro Ala Thr Ile Thr Asp Lys Val Ile Pro Ala Cys Leu
        675                 680                 685

Pro Ser Pro Asn Tyr Met Val Ala Asp Arg Thr Ile Cys Tyr Ile Thr
    690                 695                 700

Gly Trp Gly Glu Thr Gln Gly Thr Phe Gly Ala Gly Arg Leu Lys Glu
705                 710                 715                 720

Ala Gln Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Val Glu Tyr
                725                 730                 735

Leu Asn Asn Arg Val Lys Ser Thr Glu Leu Cys Ala Gly Gln Leu Ala
            740                 745                 750

Gly Gly Val Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys
        755                 760                 765

Phe Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu
    770                 775                 780

Gly Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg
785                 790                 795                 800

Phe Val Asp Trp Ile Glu Arg Glu Met Arg Asn Asn
                805                 810


<210> SEQ ID NO 36
<211> LENGTH: 812
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 36

Met Asp His Lys Glu Ile Ile Leu Leu Phe Leu Leu Phe Leu Lys Pro
1               5                   10                  15

Gly Gln Gly Asp Ser Leu Asp Gly Tyr Val Ser Thr Gln Gly Ala Ser
            20                  25                  30

Leu His Ser Leu Thr Lys Lys Gln Leu Ala Ala Gly Ser Ile Ala Asp
        35                  40                  45

Cys Leu Ala Lys Cys Glu Gly Glu Thr Asp Phe Ile Cys Arg Ser Phe
    50                  55                  60

Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Ser
65                  70                  75                  80

Lys Thr Ser Ser Ile Ile Arg Met Arg Asp Val Ile Leu Phe Glu Lys
                85                  90                  95

Arg Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Lys Gly Tyr Arg
            100                 105                 110

Gly Thr Met Ser Lys Thr Lys Thr Gly Val Thr Cys Gln Lys Trp Ser
        115                 120                 125

Asp Thr Ser Pro His Val Pro Lys Tyr Ser Pro Ser Thr His Pro Ser
    130                 135                 140

Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln
145                 150                 155                 160

Gly Pro Trp Cys Tyr Thr Thr Asp Pro Asp Gln Arg Tyr Glu Tyr Cys
                165                 170                 175

Asn Ile Pro Glu Cys Glu Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys
            180                 185                 190

Tyr Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Asp Cys Gln Ser
        195                 200                 205

Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ala Lys Phe
    210                 215                 220

Pro Ser Lys Asn Leu Lys Met Asn Tyr Cys Arg Asn Pro Asp Gly Glu
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225                 230                 235                 240

Pro Arg Pro Trp Cys Phe Thr Thr Asp Pro Asn Lys Arg Trp Glu Tyr
                245                 250                 255

Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Pro Gly Pro Thr
            260                 265                 270

Tyr Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser
        275                 280                 285

Val Thr Ala Ser Gly Lys Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro
    290                 295                 300

His Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu
305                 310                 315                 320

Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr
                325                 330                 335

Thr Thr Asp Ser Gln Leu Arg Trp Glu Tyr Cys Glu Ile Pro Ser Cys
            340                 345                 350

Gly Ser Ser Val Ser Pro Asp Gln Ser Asp Ser Ser Val Leu Pro Glu
        355                 360                 365

Gln Thr Pro Val Val Gln Glu Cys Tyr Gln Gly Asn Gly Lys Ser Tyr
    370                 375                 380

Arg Gly Thr Ser Ser Thr Thr Asn Thr Gly Lys Lys Cys Gln Ser Trp
385                 390                 395                 400

Val Ser Met Thr Pro His Ser His Ser Lys Thr Pro Ala Asn Phe Pro
                405                 410                 415

Asp Ala Gly Leu Glu Met Asn Tyr Cys Arg Asn Pro Asp Asn Asp Gln
            420                 425                 430

Arg Gly Pro Trp Cys Phe Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr
        435                 440                 445

Cys Asn Leu Lys Arg Cys Ser Glu Thr Gly Gly Gly Val Ala Glu Ser
    450                 455                 460

Ala Ile Val Pro Gln Val Pro Ser Ala Pro Gly Thr Ser Glu Thr Asp
465                 470                 475                 480

Cys Met Tyr Gly Asn Gly Lys Glu Tyr Arg Gly Lys Thr Ala Val Thr
                485                 490                 495

Ala Ala Gly Thr Pro Cys Gln Glu Trp Ala Ala Gln Glu Pro His Ser
            500                 505                 510

His Arg Ile Phe Thr Pro Gln Thr Asn Pro Arg Ala Gly Leu Glu Lys
        515                 520                 525

Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Asn Gly Pro Trp Cys Tyr
    530                 535                 540

Thr Met Asn Pro Arg Lys Leu Tyr Asp Tyr Cys Asn Ile Pro Leu Cys
545                 550                 555                 560

Ala Ser Leu Ser Ser Phe Glu Cys Gly Lys Pro Gln Val Glu Pro Lys
                565                 570                 575

Lys Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala Asn Pro His Ser
            580                 585                 590

Trp Pro Trp Gln Ile Ser Leu Arg Thr Arg Phe Ser Gly Gln His Phe
        595                 600                 605

Cys Gly Gly Thr Leu Ile Ser Pro Glu Trp Val Leu Thr Ala Ala His
    610                 615                 620

Cys Leu Glu Lys Ser Ser Arg Pro Glu Phe Tyr Lys Val Ile Leu Gly
625                 630                 635                 640

Ala His Glu Glu Arg Ile Leu Gly Ser Asp Val Gln Gln Ile Ala Val
                645                 650                 655
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Thr Lys Leu Val Leu Glu Pro Asn Asp Ala Asp Ile Ala Leu Leu Lys
            660                 665                 670

Leu Ser Arg Pro Ala Thr Ile Thr Asp Asn Val Ile Pro Ala Cys Leu
        675                 680                 685

Pro Ser Pro Asn Tyr Val Val Ala Asp Arg Thr Leu Cys Tyr Ile Thr
    690                 695                 700

Gly Trp Gly Glu Thr Lys Gly Thr Pro Gly Ala Gly Arg Leu Lys Glu
705                 710                 715                 720

Ala Gln Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Ala Glu Tyr
                725                 730                 735

Leu Asn Asn Arg Val Lys Ser Thr Glu Leu Cys Ala Gly His Leu Ala
            740                 745                 750

Gly Gly Ile Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys
        755                 760                 765

Phe Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu
    770                 775                 780

Gly Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg
785                 790                 795                 800

Tyr Val Asn Trp Ile Glu Arg Glu Met Arg Asn Asp
                805                 810


<210> SEQ ID NO 37
<211> LENGTH: 811
<212> TYPE: PRT
<213> ORGANISM: Erinaceus europaeus

<400> SEQUENCE: 37

Met Gln Arg Lys Glu Leu Val Leu Leu Phe Leu Leu Phe Leu Gln Pro
1               5                   10                  15

Gly His Gly Ile Pro Leu Asp Asp Tyr Val Thr Thr Gln Gly Ala Ser
            20                  25                  30

Leu Ser Ser Ser Thr Lys Lys Gln Leu Ser Val Gly Ser Thr Glu Glu
        35                  40                  45

Cys Ala Val Lys Cys Glu Lys Glu Thr Ser Phe Ile Cys Arg Ser Phe
    50                  55                  60

Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Ser
65                  70                  75                  80

Lys Ser Thr Pro Val Leu Arg Met Arg Asp Val Ile Leu Phe Glu Lys
                85                  90                  95

Lys Met Tyr Leu Ser Glu Cys Lys Val Gly Asn Gly Lys Tyr Tyr Arg
            100                 105                 110

Gly Thr Val Ser Lys Thr Lys Thr Gly Leu Thr Cys Gln Lys Trp Ser
        115                 120                 125

Ala Glu Thr Pro His Lys Pro Arg Phe Ser Pro Asp Glu Asn Pro Ser
    130                 135                 140

Glu Gly Leu Asp Gln Asn Tyr Cys Arg Asn Pro Asp Asn Asp Pro Lys
145                 150                 155                 160

Gly Pro Trp Cys Tyr Thr Met Asp Pro Glu Val Arg Tyr Glu Tyr Cys
                165                 170                 175

Glu Ile Ile Gln Cys Glu Asp Glu Cys Met His Cys Ser Gly Gln Asn
            180                 185                 190

Tyr Val Gly Lys Ile Ser Arg Thr Met Ser Gly Leu Glu Cys Gln Pro
        195                 200                 205

Trp Asp Ser Gln Ile Pro His Pro His Gly Phe Ile Pro Ser Lys Phe
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    210                 215                 220

Pro Ser Lys Asn Leu Lys Met Asn Tyr Cys Arg Asn Pro Asp Gly Glu
225                 230                 235                 240

Pro Arg Pro Trp Cys Phe Thr Met Asp Arg Asn Lys Arg Trp Glu Tyr
                245                 250                 255

Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Ser Gly Pro Thr
            260                 265                 270

Tyr Gln Cys Leu Met Gly Asn Gly Glu His Tyr Gln Gly Asn Val Ala
        275                 280                 285

Val Thr Val Ser Gly Leu Thr Cys Gln Arg Trp Gly Glu Gln Ser Pro
    290                 295                 300

His Arg His Asp Arg Thr Pro Glu Asn Tyr Pro Cys Lys Asn Leu Asp
305                 310                 315                 320

Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Pro Ala Pro Trp Cys Phe
                325                 330                 335

Thr Thr Asn Ser Ser Val Arg Trp Glu Phe Cys Lys Ile Pro Asp Cys
            340                 345                 350

Val Ser Ser Ala Ser Glu Thr Glu His Ser Asp Ala Pro Val Ile Val
        355                 360                 365

Pro Pro Glu Gln Thr Pro Val Val Gln Glu Cys Tyr Gln Gly Asn Gly
    370                 375                 380

Gln Ser Tyr Arg Gly Thr Ser Ser Thr Thr Ile Thr Gly Lys Lys Cys
385                 390                 395                 400

Gln Pro Trp Thr Ser Met Arg Pro His Arg His Ser Lys Thr Pro Glu
                405                 410                 415

Asn Tyr Pro Asp Ala Asp Leu Thr Met Asn Tyr Cys Arg Asn Pro Asp
            420                 425                 430

Gly Asp Lys Gly Pro Trp Cys Tyr Thr Thr Asp Pro Ser Val Arg Trp
        435                 440                 445

Glu Phe Cys Asn Leu Lys Lys Cys Ser Gly Thr Glu Met Ser Ala Thr
    450                 455                 460

Asn Ser Ser Pro Val Gln Val Ser Ser Ala Ser Glu Ser Ser Glu Gln
465                 470                 475                 480

Asp Cys Ile Ile Asp Asn Gly Lys Gly Tyr Arg Gly Thr Lys Ala Thr
                485                 490                 495

Thr Gly Ala Gly Thr Pro Cys Gln Ala Trp Ala Ala Gln Glu Pro His
            500                 505                 510

Arg His Ser Ile Phe Thr Pro Glu Thr Asn Pro Arg Ala Asp Leu Gln
        515                 520                 525

Glu Asn Tyr Cys Arg Asn Pro Asp Gly Asp Ala Asn Gly Pro Trp Cys
    530                 535                 540

Tyr Thr Thr Asn Pro Arg Lys Leu Phe Asp Tyr Cys Asp Ile Pro His
545                 550                 555                 560

Cys Val Ser Pro Ser Ser Ala Asp Cys Gly Lys Pro Lys Val Glu Pro
                565                 570                 575

Lys Lys Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala Asn Pro His
            580                 585                 590

Ser Trp Pro Trp Gln Val Ser Leu Arg Arg Phe Gly Gln His Phe Cys
        595                 600                 605

Gly Gly Thr Leu Ile Ser Pro Glu Trp Val Val Thr Ala Ala His Cys
    610                 615                 620

Leu Glu Lys Phe Ser Asn Pro Ala Ile Tyr Lys Val Val Leu Gly Ala
625                 630                 635                 640
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His Gln Glu Thr Arg Leu Glu Arg Asp Val Gln Ile Lys Gly Val Thr
                645                 650                 655

Lys Met Phe Leu Glu Pro Tyr Arg Ala Asp Ile Ala Leu Leu Lys Leu
            660                 665                 670

Ser Ser Pro Ala Ile Ile Thr Asp Lys Ile Ile Pro Ala Cys Leu Pro
        675                 680                 685

Asn Ser Asn Tyr Met Val Ala Asp Arg Ser Leu Cys Tyr Ile Thr Gly
    690                 695                 700

Trp Gly Glu Thr Lys Gly Thr Tyr Gly Ala Gly Leu Leu Lys Glu Ala
705                 710                 715                 720

Gln Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Gln Glu Leu Leu
                725                 730                 735

Asn Gly Arg Val Arg Ser Thr Glu Leu Cys Ala Gly His Leu Ala Gly
            740                 745                 750

Gly Val Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Phe
        755                 760                 765

Glu Lys Asp Arg Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu Gly
    770                 775                 780

Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg Tyr
785                 790                 795                 800

Val Ser Trp Leu Gln Asp Val Met Arg Asn Asn
                805                 810


<210> SEQ ID NO 38
<211> LENGTH: 780
<212> TYPE: PRT
<213> ORGANISM: Oryctolagus cuniculus

<400> SEQUENCE: 38

Met Glu Gln Arg Ala Val Val Leu Leu Leu Leu Leu Leu Leu Lys Pro
1               5                   10                  15

Gly Gln Ala Glu Pro Leu Asp Asp Tyr Val Asn Thr Gln Gly Ala Ser
            20                  25                  30

Leu Phe Ser Phe Thr Lys Lys Gln Leu Gly Ala Ala Ser Ile Ala Glu
        35                  40                  45

Cys Ala Ala Arg Cys Glu Ala Glu Thr Glu Phe Thr Cys Arg Ser Phe
    50                  55                  60

Gln Tyr His Ser Lys Glu Gln Gln Cys Val Val Met Ala Glu Asn Ser
65                  70                  75                  80

Lys Ser Ser Ala Ile Ile Arg Arg Arg Asp Val Val Leu Phe Glu Lys
                85                  90                  95

Arg Met Tyr Leu Ser Glu Cys Lys Ile Gly Asn Gly Arg Ser Tyr Arg
            100                 105                 110

Gly Thr Lys Ser Lys Thr Lys Thr Gly Phe Thr Cys Gln Lys Trp Ser
        115                 120                 125

Ser Ser Tyr Pro His Lys Pro Asn Phe Thr Pro Lys Lys Tyr Pro Ala
    130                 135                 140

Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln
145                 150                 155                 160

Gly Pro Trp Cys Tyr Thr Thr Asn Pro Asp Glu Arg Phe Asp Tyr Cys
                165                 170                 175

Asp Ile Pro Glu Cys Glu Asp Glu Cys Met His Cys Ser Gly Glu Asn
            180                 185                 190

Tyr Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Ile Glu Cys Gln Ala
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        195                 200                 205

Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ser Lys Phe
    210                 215                 220

Pro Asn Lys Asn Leu Lys Lys Asn Tyr Cys Arg Asn Pro Asp Gly Glu
225                 230                 235                 240

Pro Arg Pro Trp Cys Phe Thr Met Asp Pro Lys Lys Arg Trp Glu Leu
                245                 250                 255

Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Ser Gly Pro Thr
            260                 265                 270

His Gln Cys Leu Lys Gly Arg Gly Glu Ser Tyr Arg Gly Lys Val Ala
        275                 280                 285

Arg Thr Lys Ser Gly Leu Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro
    290                 295                 300

His Leu His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asp Leu Asp
305                 310                 315                 320

Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Ser Ala Pro Trp Cys Tyr
                325                 330                 335

Thr Thr Asp Ser Lys Val Arg Trp Glu His Cys Asp Ile Pro Ser Cys
            340                 345                 350

Ala Ser Ser Pro Thr Ser Val Glu Pro Leu Asp Ala Pro Ala Pro Pro
        355                 360                 365

Glu Glu Thr Pro Val Val Gln Glu Cys Tyr Gln Gly Asn Gly Gln Ser
    370                 375                 380

Tyr Arg Gly Thr Ser Ser Thr Thr Ile Thr Gly Arg Lys Cys Gln Ser
385                 390                 395                 400

Trp Leu Ser Met Thr Pro His Arg His Gln Arg Thr Pro Gln Asn Tyr
                405                 410                 415

Pro Asn Ala Asp Leu Thr Met Asn Tyr Cys Arg Asn Pro Asp Asp Asp
            420                 425                 430

Ile Arg Pro Trp Cys Tyr Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr
        435                 440                 445

Cys Asn Leu Arg Arg Cys Ser Glu Pro Ala Ala Ser Pro Ala Ala Thr
    450                 455                 460

Val Pro Thr Ala Gln Leu Pro Arg Pro Glu Ala Thr Phe Glu Pro Asp
465                 470                 475                 480

Cys Met Phe Gly Asn Gly Lys Gly Tyr Arg Gly Lys Lys Ala Thr Thr
                485                 490                 495

Ala Asp Gly Thr Pro Cys Gln Gly Trp Ala Ala Gln Glu Pro His Arg
            500                 505                 510

His Asn Ile Phe Thr Pro Glu Thr Asn Pro Arg Ala Gly Leu Glu Arg
        515                 520                 525

Asn Tyr Cys Arg Asn Pro Asp Gly Asp Thr Asn Gly Pro Trp Cys Tyr
    530                 535                 540

Thr Met Asn Pro Arg Lys Leu Tyr Asp Tyr Cys Asp Val Pro Gln Cys
545                 550                 555                 560

Ala Ser Ser Ser Ser Tyr Asp Cys Gly Lys Pro Lys Val Glu Pro Lys
                565                 570                 575

Lys Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala Asn Pro His Ser
            580                 585                 590

Trp Pro Trp Gln Ile Ser Leu Arg Thr Arg Thr Gly Gln His Phe Cys
        595                 600                 605

Gly Gly Thr Leu Ile Ala Pro Glu Trp Val Leu Thr Ala Ala His Cys
    610                 615                 620
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```
Leu Glu Lys Tyr Pro Arg Pro Ser Ala Tyr Arg Val Ile Leu Gly Ala
625                 630                 635                 640

His Lys Glu Val Asn Leu Glu Leu Asp Val Gln Asp Ile Asp Val Ala
                645                 650                 655

Lys Leu Phe Leu Glu Pro Ser Arg Ala Asp Ile Ala Leu Met Lys Leu
            660                 665                 670

Ser Ser Leu Glu Trp Ala Trp Thr Tyr Gly Ala Gly Leu Leu Lys Glu
        675                 680                 685

Ala Gln Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Phe Glu Tyr
    690                 695                 700

Leu Asn Gly Arg Val Arg Ser Thr Glu Leu Cys Ala Gly His Leu Ala
705                 710                 715                 720

Gly Gly Thr Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys
                725                 730                 735

Phe Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu
            740                 745                 750

Gly Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg
        755                 760                 765

Phe Val Asp Trp Ile Glu Arg Thr Met Arg Asn Asn
    770                 775                 780


<210> SEQ ID NO 39
<211> LENGTH: 827
<212> TYPE: PRT
<213> ORGANISM: Pan troglodytes

<400> SEQUENCE: 39

Met Glu His Lys Glu Val Val Leu Leu Leu Leu Leu Phe Leu Lys Ser
1               5                   10                  15

Gly Gln Gly Glu Pro Leu Asp Asp Tyr Val Asn Thr Gln Gly Ala Ser
            20                  25                  30

Leu Phe Ser Val Thr Lys Lys Gln Leu Gly Ala Gly Ser Ile Glu Glu
        35                  40                  45

Cys Ala Ala Lys Cys Glu Glu Asp Lys Glu Phe Thr Cys Arg Tyr Phe
    50                  55                  60

His Cys Arg Cys Thr Tyr Pro Glu Ile Cys Asn Ser Asp Gly Lys Ala
65                  70                  75                  80

Phe Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn
                85                  90                  95

Arg Lys Ser Ser Ile Ile Ile Arg Met Arg Asp Val Val Leu Phe Glu
            100                 105                 110

Lys Lys Val Tyr Leu Ser Glu Cys Lys Thr Gly Asn Gly Lys Asn Tyr
        115                 120                 125

Arg Gly Thr Met Ser Lys Thr Lys Asn Gly Ile Thr Cys Gln Lys Trp
    130                 135                 140

Ser Ser Thr Ser Pro His Arg Pro Arg Phe Ser Pro Ala Thr His Pro
145                 150                 155                 160

Ser Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Pro
                165                 170                 175

Gln Gly Pro Trp Cys Tyr Thr Thr Asp Pro Glu Lys Arg Tyr Asp Tyr
            180                 185                 190

Cys Asp Ile Leu Glu Cys Glu Glu Glu Cys Met His Cys Ser Gly Glu
        195                 200                 205

Asn Tyr Asp Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Glu Cys Gln
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```
    210                 215                 220

Ala Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ser Lys
225                 230                 235                 240

Phe Pro Asn Lys Asn Leu Lys Lys Asn Tyr Cys Arg Asn Pro Asp Gly
                245                 250                 255

Glu Leu Arg Pro Trp Cys Phe Thr Thr Asp Pro Asn Lys Arg Trp Glu
            260                 265                 270

Leu Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Ser Ser Gly Pro
        275                 280                 285

Thr Tyr Gln Cys Leu Lys Gly Thr Gly Glu Asn Tyr Arg Gly Asn Val
    290                 295                 300

Ala Val Thr Val Ser Gly His Thr Cys Gln His Trp Ser Ala Gln Thr
305                 310                 315                 320

Pro His Thr His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu
                325                 330                 335

Asp Glu Asn Tyr Cys Arg Asn Pro Asp Gly Lys Arg Ala Pro Trp Cys
            340                 345                 350

His Thr Thr Asn Ser Gln Val Arg Trp Glu Tyr Cys Lys Ile Pro Ser
        355                 360                 365

Cys Asp Ser Ser Leu Val Ser Thr Glu Gln Leu Ala Pro Thr Ala Pro
    370                 375                 380

Pro Glu Leu Thr Pro Val Val Gln Asp Cys Tyr His Gly Asp Gly Gln
385                 390                 395                 400

Ser Tyr Arg Gly Thr Ser Ser Thr Thr Thr Thr Gly Lys Lys Cys Gln
                405                 410                 415

Ser Trp Ser Ser Met Thr Pro His Arg His Gln Lys Thr Pro Glu Asn
            420                 425                 430

Tyr Pro Asn Ala Gly Leu Thr Met Asn Tyr Cys Arg Asn Pro Asp Ala
        435                 440                 445

Asp Lys Gly Pro Trp Cys Phe Thr Thr Asp Pro Ser Val Arg Trp Glu
    450                 455                 460

Tyr Cys Asn Leu Lys Lys Cys Ser Gly Thr Glu Ala Ser Val Val Ala
465                 470                 475                 480

Pro Pro Pro Val Val Gln Leu Pro Asn Val Glu Thr Pro Ser Glu Glu
                485                 490                 495

Asp Cys Met Phe Gly Asn Gly Lys Gly Tyr Arg Gly Lys Arg Ala Thr
            500                 505                 510

Thr Val Thr Gly Thr Pro Cys Gln Asp Trp Ala Ala Gln Glu Pro His
        515                 520                 525

Arg His Ser Ile Phe Thr Pro Glu Thr Asn Pro Arg Ala Gly Leu Glu
    530                 535                 540

Lys Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Gly Gly Pro Trp Cys
545                 550                 555                 560

Tyr Thr Thr Asn Pro Arg Lys Leu Tyr Asp Tyr Cys Asp Val Pro Gln
                565                 570                 575

Cys Ala Ser Pro Ser Phe Asp Cys Gly Lys Pro Gln Val Glu Pro Lys
            580                 585                 590

Lys Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala His Pro His Ser
        595                 600                 605

Trp Pro Trp Gln Val Ser Leu Arg Thr Arg Leu Gly Met His Phe Cys
    610                 615                 620

Gly Gly Thr Leu Ile Ser Pro Glu Trp Val Leu Thr Ala Ala His Cys
625                 630                 635                 640
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```
Leu Glu Lys Ser Pro Arg Pro Ser Ser Tyr Lys Val Ile Leu Gly Ala
                645                 650                 655

His Gln Glu Val Lys Leu Glu Pro His Val Gln Glu Ile Glu Val Ser
            660                 665                 670

Arg Leu Phe Leu Glu Pro Thr Arg Thr Asp Ile Ala Leu Leu Lys Leu
        675                 680                 685

Ser Ser Pro Ala Ile Ile Thr Asp Lys Val Ile Pro Ala Cys Leu Pro
    690                 695                 700

Ser Pro Asn Tyr Val Val Ala Asp Arg Thr Glu Cys Phe Ile Thr Gly
705                 710                 715                 720

Trp Gly Glu Thr Gln Gly Thr Phe Gly Ala Gly Leu Leu Lys Glu Ala
                725                 730                 735

Gln Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Asn Glu Phe Leu
            740                 745                 750

Asn Gly Arg Val Lys Ser Thr Glu Leu Cys Ala Gly His Leu Ala Gly
        755                 760                 765

Gly Thr Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Phe
    770                 775                 780

Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu Gly
785                 790                 795                 800

Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg Phe
                805                 810                 815

Val Thr Trp Ile Glu Gly Val Met Arg Asn Asn
            820                 825


<210> SEQ ID NO 40
<211> LENGTH: 769
<212> TYPE: PRT
<213> ORGANISM: Ailuropoda melanoleuca

<400> SEQUENCE: 40

Phe Val Arg Arg Ser Phe Glu Tyr His Ser Lys Glu Gln Gln Cys Ala
1               5                   10                  15

Ile Met Ala Glu Asn Ser Lys Ser Ser Ala Val Phe Arg Met Arg Asp
            20                  25                  30

Val Ile Leu Phe Gln Lys Arg Ile Tyr Leu Ser Glu Cys Lys Thr Gly
        35                  40                  45

Asn Gly Lys Thr Tyr Arg Gly Thr Met Ser Lys Thr Lys Asn Gly Val
    50                  55                  60

Ala Cys Gln Lys Trp Ser Asp Thr Phe Pro His Lys Pro Asn Tyr Thr
65                  70                  75                  80

Pro Glu Lys His Pro Leu Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn
                85                  90                  95

Pro Asp Asn Asp Glu Lys Gly Pro Trp Cys Tyr Thr Thr Asp Pro Asn
            100                 105                 110

Gln Arg Phe Asp Tyr Cys Ser Ile Pro Gln Cys Glu Asp Glu Cys Met
        115                 120                 125

His Cys Ser Gly Glu Asn Tyr Glu Gly Lys Val Ser Lys Thr Lys Ser
    130                 135                 140

Gly Leu Glu Cys Gln Ala Trp Asn Ser Gln Thr Pro His Ala His Gly
145                 150                 155                 160

Tyr Ile Pro Ser Lys Phe Pro Asn Lys Asn Leu Lys Met Asn Tyr Cys
                165                 170                 175

Arg Asn Pro Asp Gly Glu Pro Arg Pro Trp Cys Phe Thr Met Asp Pro
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            180                 185                 190

Asn Lys Arg Trp Glu Phe Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro
        195                 200                 205

Pro Pro Ser Gly Pro Thr Tyr Gln Cys Leu Lys Gly Lys Gly Glu Asn
    210                 215                 220

Tyr Arg Gly Lys Val Ser Val Thr Ala Ser Gly His Thr Cys Gln Arg
225                 230                 235                 240

Trp Ser Glu Gln Thr Pro His Lys His Asn Arg Thr Pro Glu Asn Phe
                245                 250                 255

Pro Cys Lys Asn Leu Asp Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu
            260                 265                 270

Ser Ala Pro Trp Cys Tyr Thr Thr Asp Ser Glu Val Arg Trp Glu His
        275                 280                 285

Cys Ser Ile Pro Ser Cys Glu Ser Ser Pro Leu Thr Leu Asp Ser Leu
    290                 295                 300

Asp Thr Pro Ala Ser Ile Pro Pro Glu Gln Thr Pro Val Val Gln Glu
305                 310                 315                 320

Cys Tyr Gln Gly Asn Gly Gln Thr Tyr Arg Gly Thr Ser Ser Thr Thr
                325                 330                 335

Ile Thr Gly Lys Lys Cys Gln Pro Trp Ser Ser Met Ser Pro His Arg
            340                 345                 350

His Glu Lys Thr Pro Glu Arg Phe Pro Asn Ala Gly Leu Thr Met Asn
        355                 360                 365

Tyr Cys Arg Asn Pro Asp Gly Asp Lys Ser Pro Trp Cys Tyr Thr Thr
    370                 375                 380

Asp Pro Ser Val Arg Trp Glu Phe Cys Asn Leu Lys Lys Cys Leu Asp
385                 390                 395                 400

Thr Glu Glu Ser Gly Thr Ser Ser Pro Thr Val Pro Gln Val Pro Ser
                405                 410                 415

Gly Glu Glu Pro Ser Glu Thr Asp Cys Met Phe Gly Asn Gly Lys Gly
            420                 425                 430

Tyr Arg Gly Lys Lys Ala Thr Thr Val Leu Gly Ile Pro Cys Gln Glu
        435                 440                 445

Trp Thr Ala Gln Glu Pro His Lys His Ser Ile Phe Thr Pro Glu Thr
    450                 455                 460

Asn Pro Arg Ala Glu His Leu Leu Cys Pro Thr Cys Leu Val Pro Ser
465                 470                 475                 480

Val Pro Thr Val Phe Phe Phe Phe Phe Phe Phe Phe Leu Phe Leu Asp
                485                 490                 495

Val Asn Gly Pro Trp Cys Tyr Thr Thr Asn Pro Arg Lys Leu Phe Asp
            500                 505                 510

Tyr Cys Asp Ile Pro Gln Cys Ala Ser Gly Ser Phe Asp Cys Gly Lys
        515                 520                 525

Pro Gln Val Glu Pro Lys Lys Cys Pro Gly Arg Val Val Gly Gly Cys
    530                 535                 540

Val Ala Asn Pro His Ser Trp Pro Trp Gln Ile Ser Leu Arg Thr Arg
545                 550                 555                 560

Phe Gly Gln His Phe Cys Gly Gly Thr Leu Ile Ser Pro Glu Trp Val
                565                 570                 575

Leu Thr Ala Ala His Cys Leu Glu Arg Ser Pro Arg Pro Ala Ala Tyr
            580                 585                 590

Lys Val Ile Leu Gly Ala His Arg Glu Phe Asn Leu Glu Ser Asp Val
        595                 600                 605
```

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Gln Glu Ile Glu Val Ser Lys Leu Phe Leu Glu Pro Thr His Ala Asp
    610                 615                 620

Ile Ala Leu Ile Lys Leu Gln Ser Pro Ala Val Leu Thr Ser Lys Val
625                 630                 635                 640

Ile Pro Ala Cys Leu Pro Ser Pro Asn Tyr Val Val Ala Asp Arg Thr
                645                 650                 655

Leu Cys Tyr Ile Thr Gly Trp Gly Glu Thr Gln Gly Thr Phe Gly Val
            660                 665                 670

Gly Leu Leu Lys Glu Ala Gln Leu Pro Val Ile Glu Asn Lys Val Cys
        675                 680                 685

Asn Arg Tyr Glu Tyr Leu Asn Gly Lys Val Lys Ser Thr Glu Leu Cys
    690                 695                 700

Ala Gly Asn Leu Ala Gly Gly Thr Asp Ser Cys Gln Gly Asp Ser Gly
705                 710                 715                 720

Gly Pro Leu Val Cys Phe Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val
                725                 730                 735

Thr Ser Trp Gly Leu Gly Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr
            740                 745                 750

Val Arg Val Ser Arg Phe Val Thr Trp Ile Glu Glu Ile Met Arg Asn
        755                 760                 765

Asn


&lt;210&gt; SEQ ID NO 41
&lt;211&gt; LENGTH: 334
&lt;212&gt; TYPE: PRT
&lt;213&gt; ORGANISM: Papio hamadryas

&lt;400&gt; SEQUENCE: 41

Ile Arg Leu Asp Cys Met Phe Gly Asn Gly Lys Arg Tyr Arg Gly Lys
1               5                   10                  15

Lys Ala Thr Thr Val Thr Gly Thr Pro Cys Gln Glu Trp Ala Ala Lys
            20                  25                  30

Glu Pro His Ser His Leu Ile Phe Thr Pro Glu Thr Tyr Pro Arg Ala
        35                  40                  45

Gly Leu Glu Lys Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Gly Gly
    50                  55                  60

Pro Trp Cys Tyr Thr Thr Asn Pro Arg Lys Leu Tyr Asp Tyr Cys Asp
65                  70                  75                  80

Val Pro Gln Cys Ala Ser Ser Ser Phe Asp Cys Gly Lys Pro Gln Val
                85                  90                  95

Glu Pro Lys Lys Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala His
            100                 105                 110

Ala His Ser Trp Pro Trp Gln Val Ser Leu Arg Thr Arg Phe Gly Met
        115                 120                 125

His Phe Cys Gly Gly Thr Leu Ile Ser Pro Glu Trp Val Leu Thr Ala
    130                 135                 140

Ala His Cys Leu Glu Lys Ser Pro Arg Pro Ser Phe Tyr Lys Val Ile
145                 150                 155                 160

Leu Gly Ala His Gln Glu Val Arg Leu Glu Pro His Val Gln Glu Ile
                165                 170                 175

Glu Val Ser Lys Met Phe Ser Glu Pro Ala Gly Ala Asp Ile Ala Leu
            180                 185                 190

Leu Lys Leu Ser Ser Pro Ala Ile Ile Thr Asp Lys Val Ile Pro Ala
        195                 200                 205
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Cys Leu Pro Ser Pro Asn Tyr Val Val Ala Asp Arg Thr Glu Cys Phe
    210                 215                 220

Ile Thr Gly Trp Gly Glu Thr Gln Gly Thr Tyr Gly Ala Gly Leu Leu
225                 230                 235                 240

Lys Glu Ala Arg Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Tyr
                245                 250                 255

Glu Phe Leu Asn Gly Arg Val Lys Ser Thr Glu Leu Cys Ala Gly His
            260                 265                 270

Leu Ala Gly Gly Thr Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu
        275                 280                 285

Val Cys Phe Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp
    290                 295                 300

Gly Leu Gly Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val
305                 310                 315                 320

Ser Arg Phe Val Thr Trp Ile Glu Gly Val Met Arg Asn Asn
                325                 330


<210> SEQ ID NO 42
<211> LENGTH: 343
<212> TYPE: PRT
<213> ORGANISM: Ovis aries

<400> SEQUENCE: 42

Ala Pro Gln Ala Pro Ser Val Glu Asn Pro Pro Glu Ala Asp Cys Met
1               5                   10                  15

Leu Gly Ile Gly Lys Gly Tyr Arg Gly Lys Lys Ala Thr Thr Val Ala
            20                  25                  30

Gly Val Pro Cys Gln Glu Trp Ala Ala Gln Glu Pro His Arg His Gly
        35                  40                  45

Ile Phe Thr Pro Glu Thr Asn Pro Arg Ala Gly Leu Glu Lys Asn Tyr
    50                  55                  60

Cys Arg Asn Pro Asp Gly Asp Val Asn Gly Pro Trp Cys Tyr Thr Thr
65                  70                  75                  80

Asn Pro Arg Lys Leu Phe Asp Tyr Cys Asp Ile Pro Gln Cys Glu Ser
                85                  90                  95

Ser Phe Asp Cys Gly Lys Pro Lys Val Glu Pro Lys Lys Cys Pro Ala
            100                 105                 110

Arg Val Val Gly Gly Cys Val Ala Thr Pro His Ser Trp Pro Trp Gln
        115                 120                 125

Val Ser Leu Arg Arg Arg Ser Arg Glu His Phe Cys Gly Gly Thr Leu
    130                 135                 140

Ile Ser Pro Glu Trp Val Leu Thr Ala Ala His Cys Leu Asp Ser Ile
145                 150                 155                 160

Leu Gly Pro Ser Phe Tyr Thr Val Ile Leu Gly Ala His Tyr Glu Met
                165                 170                 175

Ala Arg Glu Ala Ser Val Gln Glu Ile Pro Val Ser Arg Leu Phe Leu
            180                 185                 190

Glu Pro Ser Arg Ala Asp Ile Ala Leu Leu Lys Leu Ser Ser Pro Ala
        195                 200                 205

Val Ile Thr Asp Glu Val Ile Pro Ala Cys Leu Pro Ser Pro Asn Tyr
    210                 215                 220

Val Val Ala Asp Lys Thr Val Cys Tyr Ile Thr Gly Trp Gly Glu Thr
225                 230                 235                 240

Gln Gly Thr Phe Gly Val Gly Arg Leu Lys Glu Ala Arg Leu Pro Val
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                245                 250                 255

Ile Glu Asn Lys Val Cys Asn Arg Tyr Glu Tyr Leu Asn Gly Arg Val
            260                 265                 270

Lys Ser Thr Glu Leu Cys Ala Gly Asp Leu Ala Gly Gly Thr Asp Ser
        275                 280                 285

Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Phe Glu Lys Asp Lys
    290                 295                 300

Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu Gly Cys Ala Arg Pro
305                 310                 315                 320

Asn Lys Pro Gly Val Tyr Val Arg Val Ser Thr Tyr Val Pro Trp Ile
                325                 330                 335

Glu Glu Thr Met Arg Arg Tyr
            340


<210> SEQ ID NO 43
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      peptide

<400> SEQUENCE: 43

Ala Pro Ser Phe Asp Cys Gly Lys Pro Gln
1               5                   10


<210> SEQ ID NO 44
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      peptide

<400> SEQUENCE: 44

Val Val Gly Gly Cys Val Ala His Pro
1               5


<210> SEQ ID NO 45
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      peptide

<400> SEQUENCE: 45

Glu Ala Gln Leu Pro Val Ile Glu Asn Lys
1               5                   10


<210> SEQ ID NO 46
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      peptide

<400> SEQUENCE: 46

Val Cys Asn Arg Tyr Glu Phe Leu Asn Gly
1               5                   10


<210> SEQ ID NO 47
<211> LENGTH: 4
```

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      peptide

<400> SEQUENCE: 47

Val Val Gly Gly
1


<210> SEQ ID NO 48
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      peptide

<400> SEQUENCE: 48

Val Gln Ser Thr Glu Leu
1               5


<210> SEQ ID NO 49
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      peptide

<400> SEQUENCE: 49

Leu Glu Lys Arg
1


<210> SEQ ID NO 50
<211> LENGTH: 810
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 50

Met Glu His Lys Glu Val Val Leu Leu Leu Leu Leu Phe Leu Lys Ser
1               5                   10                  15

Gly Gln Gly Glu Pro Leu Asp Asp Tyr Val Asn Thr Gln Gly Ala Ser
            20                  25                  30

Leu Phe Ser Val Thr Lys Lys Gln Leu Gly Ala Gly Ser Ile Glu Glu
        35                  40                  45

Cys Ala Ala Lys Cys Glu Glu Asp Glu Glu Phe Thr Cys Arg Ala Phe
    50                  55                  60

Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Arg
65                  70                  75                  80

Lys Ser Ser Ile Ile Ile Arg Met Arg Asp Val Val Leu Phe Glu Lys
                85                  90                  95

Lys Val Tyr Leu Ser Glu Cys Lys Thr Gly Asn Gly Lys Asn Tyr Arg
            100                 105                 110

Gly Thr Met Ser Lys Thr Lys Asn Gly Ile Thr Cys Gln Lys Trp Ser
        115                 120                 125

Ser Thr Ser Pro His Arg Pro Arg Phe Ser Pro Ala Thr His Pro Ser
    130                 135                 140

Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Pro Gln
145                 150                 155                 160

Gly Pro Trp Cys Tyr Thr Thr Asp Pro Glu Lys Arg Tyr Asp Tyr Cys
                165                 170                 175
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Asp Ile Leu Glu Cys Glu Glu Glu Cys Met His Cys Ser Gly Glu Asn
            180                 185                 190

Tyr Asp Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Glu Cys Gln Ala
        195                 200                 205

Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ser Lys Phe
    210                 215                 220

Pro Asn Lys Asn Leu Lys Lys Asn Tyr Cys Arg Asn Pro Asp Arg Glu
225                 230                 235                 240

Leu Arg Pro Trp Cys Phe Thr Thr Asp Pro Asn Lys Arg Trp Glu Leu
                245                 250                 255

Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Ser Ser Gly Pro Thr
            260                 265                 270

Tyr Gln Cys Leu Lys Gly Thr Gly Glu Asn Tyr Arg Gly Asn Val Ala
        275                 280                 285

Val Thr Val Ser Gly His Thr Cys Gln His Trp Ser Ala Gln Thr Pro
    290                 295                 300

His Thr His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Asp
305                 310                 315                 320

Glu Asn Tyr Cys Arg Asn Pro Asp Gly Lys Arg Ala Pro Trp Cys His
                325                 330                 335

Thr Thr Asn Ser Gln Val Arg Trp Glu Tyr Cys Lys Ile Pro Ser Cys
            340                 345                 350

Asp Ser Ser Pro Val Ser Thr Glu Gln Leu Ala Pro Thr Ala Pro Pro
        355                 360                 365

Glu Leu Thr Pro Val Val Gln Asp Cys Tyr His Gly Asp Gly Gln Ser
    370                 375                 380

Tyr Arg Gly Thr Ser Ser Thr Thr Thr Thr Gly Lys Lys Cys Gln Ser
385                 390                 395                 400

Trp Ser Ser Met Thr Pro His Arg His Gln Lys Thr Pro Glu Asn Tyr
                405                 410                 415

Pro Asn Ala Gly Leu Thr Met Asn Tyr Cys Arg Asn Pro Asp Ala Asp
            420                 425                 430

Lys Gly Pro Trp Cys Phe Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr
        435                 440                 445

Cys Asn Leu Lys Lys Cys Ser Gly Thr Glu Ala Ser Val Val Ala Pro
    450                 455                 460

Pro Pro Val Val Leu Leu Pro Asp Val Glu Thr Pro Ser Glu Glu Asp
465                 470                 475                 480

Cys Met Phe Gly Asn Gly Lys Gly Tyr Arg Gly Lys Arg Ala Thr Thr
                485                 490                 495

Val Thr Gly Thr Pro Cys Gln Asp Trp Ala Ala Gln Glu Pro His Arg
            500                 505                 510

His Ser Ile Phe Thr Pro Glu Thr Asn Pro Arg Ala Gly Leu Glu Lys
        515                 520                 525

Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Gly Gly Pro Trp Cys Tyr
    530                 535                 540

Thr Thr Asn Pro Arg Lys Leu Tyr Asp Tyr Cys Asp Val Pro Gln Cys
545                 550                 555                 560

Ala Ala Pro Ser Phe Asp Cys Gly Lys Pro Gln Val Glu Pro Lys Lys
                565                 570                 575

Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala His Pro His Ser Trp
            580                 585                 590
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Pro Trp Gln Val Ser Leu Arg Thr Arg Phe Gly Met His Phe Cys Gly
        595                 600                 605

Gly Thr Leu Ile Ser Pro Glu Trp Val Leu Thr Ala Ala His Cys Leu
    610                 615                 620

Glu Lys Ser Pro Arg Pro Ser Ser Tyr Lys Val Ile Leu Gly Ala His
625                 630                 635                 640

Gln Glu Val Asn Leu Glu Pro His Val Gln Glu Ile Glu Val Ser Arg
                645                 650                 655

Leu Phe Leu Glu Pro Thr Arg Lys Asp Ile Ala Leu Leu Lys Leu Ser
            660                 665                 670

Ser Pro Ala Val Ile Thr Asp Lys Val Ile Pro Ala Cys Leu Pro Ser
        675                 680                 685

Pro Asn Tyr Val Val Ala Asp Arg Thr Glu Cys Phe Ile Thr Gly Trp
    690                 695                 700

Gly Glu Thr Gln Gly Thr Phe Gly Ala Gly Leu Leu Lys Glu Ala Gln
705                 710                 715                 720

Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Tyr Glu Phe Leu Asn
                725                 730                 735

Gly Arg Val Gln Ser Thr Glu Leu Cys Ala Gly His Leu Ala Gly Gly
            740                 745                 750

Thr Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Phe Glu
        755                 760                 765

Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu Gly Cys
    770                 775                 780

Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg Phe Val
785                 790                 795                 800

Thr Trp Ile Glu Gly Val Met Arg Asn Asn
                805                 810
```

The invention claimed is:

1. A proteolytically active or reversibly inactivated plasmin variant comprising a heavy chain and a light chain, wherein the heavy chain comprises amino acids 543-561 of SEQ ID NO:1 and the light chain comprises amino acids 562-791 of SEQ ID NO:1, with the exception that the light chain contains:
   - (a) an amino acid other than lysine at position 698 of SEQ ID NO:1;
   - (b) an amino acid other than lysine at position 708 of SEQ ID NO:1; or
   - (c) an amino acid other than arginine, alanine, or glutamate at position 719 of SEQ ID NO:1.

2. The plasmin variant of claim **1**, wherein the light chain contains an amino acid other than lysine at position 698 of SEQ ID NO:1.

3. The plasmin variant of claim **2**, wherein the amino acid at position 698 of SEQ ID NO:1 of the light chain is Ala, Glu, Phe, His, Ile, Met, Gln or Arg.

4. The plasmin variant of claim **1**, wherein the light chain contains an amino acid other than lysine at position 708 of SEQ ID NO:1.

5. The plasmin variant of claim **4**, wherein the amino acid at position 708 of SEQ ID NO:1 of the light chain is Ala, Glu, Gln, His, Ile or Phe.

6. The plasmin variant of claim **1**, wherein the light chain contains an amino acid other than arginine, alanine, or glutamate at position 719 of SEQ ID NO:1.

7. The plasmin variant of claim **6**, wherein the amino acid at position 719 of SEQ ID NO:1 of the light chain is Gln, Ile, Phe or His.

8. The plasmin variant of claim **1**, having an autolysis constant that is at most 80% of a wild-type human plasmin autolysis constant.

9. The plasmin variant of claim **1**, having an autolysis constant that is at most 50% of a wild-type human plasmin autolysis constant.

10. The plasmin variant of claim **1**, having an autolysis constant that is at most 25% of a wild-type human plasmin autolysis constant.

11. The plasmin variant of claim **1**, having an autolysis constant that is at most 1% of a wild-type human plasmin autolysis constant.

12. The plasmin variant of claim **1**, wherein the plasmin variant is a Glu-plasmin variant, a Lys-plasmin variant, a midiplasmin variant, a miniplasmin variant, a microplasmin variant, or a delta-plasmin variant.

13. A proteolytically active or reversibly inactivated plasmin variant comprising a heavy chain and a light chain, wherein the heavy chain comprises amino acids 543-561 of SEQ ID NO:1 and the light chain comprises amino acids 562-791 of SEQ ID NO:1, with the exception that the light chain contains:
   - (a) an amino acid other than lysine at position 698 of SEQ ID NO:1 and an amino acid other than lysine at position 708 of SEQ ID NO:1;
   - (b) an amino acid other than lysine at position 698 of SEQ ID NO:1, an amino acid other than lysine at position 708 of SEQ ID NO:1, and an amino acid other than arginine at position 719 of SEQ ID NO:1;

(c) an amino acid other than lysine at position 698 of SEQ ID NO:1 and an amino acid other than arginine at position R15811719 of SEQ ID NO:1; or

(d) an amino acid other than lysine at position 708 of SEQ ID NO:1 and an amino acid other than arginine at position 719 of SEQ ID NO:1.

14. The plasmin variant of claim **13**, wherein the light chain contains an amino acid other than lysine at position 698 of SEQ ID NO:1 and an amino acid other than lysine at position 708 of SEQ ID NO:1.

15. The plasmin variant of claim **13**, wherein the light chain contains an amino acid other than lysine at position 698 of SEQ ID NO:1, an amino acid other than lysine at position 708 of SEQ ID NO:1, and an amino acid other than arginine at position 719 of SEQ ID NO:1.

16. The plasmin variant of claim **13**, wherein the light chain contains an amino acid other than lysine at position 698 of SEQ ID NO:1 and an amino acid other than arginine at position 719 of SEQ ID NO:1.

17. The plasmin variant of claim **13**, wherein the light chain contains an amino acid other than lysine at position 708 of SEQ ID NO:1 and an amino acid other than arginine at position 719 of SEQ ID NO:1.

18. The plasmin variant of claim **13**, wherein the plasmin variant is a Glu-plasmin variant, a Lys-plasmin variant, a midiplasmin variant, a miniplasmin variant, a microplasmin variant, or a delta-plasmin variant.

19. An activatable plasminogen variant comprising amino acids 543-791 of SEQ ID NO:1, with the exception that the catalytic domain contains:

(a) an amino acid other than lysine at position 698 of SEQ ID NO:1;

(b) an amino acid other than lysine at position 708 of SEQ ID NO:1;

(c) an amino acid other than arginine, alanine, or glutamate at position 719 of SEQ ID NO:1;

(d) an amino acid other than lysine at position 698 of SEQ ID NO:1 and an amino acid other than lysine at position 708 of SEQ ID NO:1;

(e) an amino acid other than lysine at position 698 of SEQ ID NO:1, an amino acid other than lysine at position 708 of SEQ ID NO:1, and an amino acid other than arginine at position 719 of SEQ ID NO:1;

(f) an amino acid other than lysine at position 698 of SEQ ID NO:1 and an amino acid other than arginine at position 719 of SEQ ID NO:1; or

(g) an amino acid other than lysine at position 708 of SEQ ID NO:1 and an amino acid other than arginine at position 719 of SEQ ID NO:1.

20. The plasminogen variant of claim **19**, wherein the plasminogen variant is a Glu-plasminogen variant, a Lys-plasminogen variant, a midiplasminogen variant, a miniplasminogen variant, a microplasminogen variant, or a delta-plasminogen variant.

21. The plasminogen variant of claim **19**, wherein the catalytic domain contains:

(a) glutamine at position 698 of SEQ ID NO:1;

(b) histidine at position 708 of SEQ ID NO:1;

(c) histidine at position 719 of SEQ ID NO:1; or

(d) glutamine at position 698 of SEQ ID NO:1, histidine at position 708 of SEQ ID NO:1, and histidine at position 719 of SEQ ID NO:1.

22. A pharmaceutical composition comprising a proteolytically active or reversibly inactivated plasmin variant comprising a heavy chain and a light chain, wherein the heavy chain comprises amino acids 543-561 of SEQ ID NO:1 and the light chain comprises amino acids 562-791 of SEQ ID NO:1, with the exception that the light chain contains:

(a) an amino acid other than lysine at position 698 of SEQ ID NO:1;

(b) an amino acid other than lysine at position 708 of SEQ ID NO:1; or

(c) an amino acid other than arginine, alanine, or glutamate at position 719 of SEQ ID NO:1;

(d) an amino acid other than lysine at position 698 of SEQ ID NO:1 and an amino acid other than lysine at position 708 of SEQ ID NO:1;

(e) an amino acid other than lysine at position 698 of SEQ ID NO:1, an amino acid other than lysine at position 708 of SEQ ID NO:1, and an amino acid other than arginine at position 719 of SEQ ID NO:1;

(f) an amino acid other than lysine at position 698 of SEQ ID NO:1 and an amino acid other than arginine at position 719 of SEQ ID NO:1; or

(g) an amino acid other than lysine at position 708 of SEQ ID NO:1 and an amino acid other than arginine at position 719 of SEQ ID NO:1, and

a pharmaceutically acceptable diluent, carrier, or adjuvant.

23. A pharmaceutical composition comprising an activatable plasminogen variant comprising amino acids 543-791 of SEQ ID NO:1, with the exception that the catalytic domain contains:

(a) an amino acid other than lysine at position 698 of SEQ ID NO:1;

(b) an amino acid other than lysine at position 708 of SEQ ID NO:1;

(c) an amino acid other than arginine, alanine, or glutamate at position 719 of SEQ ID NO:1;

(d) an amino acid other than lysine at position 698 of SEQ ID NO:1 and an amino acid other than lysine at position 708 of SEQ ID NO:1;

(e) an amino acid other than lysine at position 698 of SEQ ID NO:1, an amino acid other than lysine at position 708 of SEQ ID NO:1, and an amino acid other than arginine at position 719 of SEQ ID NO:1;

(f) an amino acid other than lysine at position 698 of SEQ ID NO:1 and an amino acid other than arginine at position 719 of SEQ ID NO:1; or

(g) an amino acid other than lysine at position 708 of SEQ ID NO:1 and an amino acid other than arginine at position 719 of SEQ ID NO:1, and

a pharmaceutically acceptable diluent, carrier, or adjuvant.

24. The pharmaceutical composition of claim **22**, further comprising an anticoagulant, a thrombolytic agent, an anti-inflammatory agent, an antiviral agent, an antibacterial agent, an antifungal agent, an anti-angiogenic agent, an anti-mitotic agent, an antihistamine, or an anesthetic.

25. The pharmaceutical composition of claim **23**, further comprising an anticoagulant, a thrombolytic agent, an anti-inflammatory agent, an antiviral agent, an antibacterial agent, an antifungal agent, an anti-angiogenic agent, an anti-mitotic agent, an antihistamine, or an anesthetic.

26. A method of promoting lysis of a pathological fibrin deposit in a human subject in need thereof, comprising administering to the human subject an effective amount of the pharmaceutical composition of claim **22**.

27. A method of promoting lysis of a pathological fibrin deposit in a human subject in need thereof, comprising administering to the human subject an effective amount of the pharmaceutical composition of claim **23**.

28. A method of inducing posterior vitreous detachment in an eye in a human subject in need thereof, the method comprising administering to the eye of the human subject an effective amount of the pharmaceutical composition of claim **22**.

29. A method of inducing posterior vitreous detachment in an eye in a human subject in need thereof, the method comprising administering to the eye of the human subject an effective amount of the pharmaceutical composition of claim **23**.

30. A proteolytically active or reversibly inactivated plasmin variant comprising a mammalian plasmin light chain amino acid sequence, with:

(a) an amino acid other than lysine or arginine at the position corresponding to position 698 of SEQ ID NO:1;
(b) an amino acid other than lysine or arginine at the position corresponding to position 708 of SEQ ID NO:1;
(c) an amino acid other than lysine, arginine, alanine, or glutamate at the position corresponding to position 719 of SEQ ID NO:1;
(d) an amino acid other than lysine or arginine at the position corresponding to position 698 of SEQ ID NO:1 and an amino acid other than lysine or arginine at the position corresponding to position 708 of SEQ ID NO:1;
(e) an amino acid other than lysine or arginine at the position corresponding to position 698 of SEQ ID NO:1, an amino acid other than lysine or arginine at the position corresponding to position 708 of SEQ ID NO:1, and an amino acid other than lysine or arginine at the position corresponding to position 719 of SEQ ID NO:1;
(f) an amino acid other than lysine or arginine at the position corresponding to position 698 of SEQ ID NO:1 and an amino acid other than lysine or arginine at the position corresponding to position 719 of SEQ ID NO:1; or
(g) an amino acid other than lysine or arginine at the position corresponding to position 708 of SEQ ID NO:1 and an amino acid other than lysine or arginine at the position corresponding to position 719 of SEQ ID NO:1.

31. An activatable plasminogen variant comprising a mammalian plasminogen amino acid sequence, with the exception that the catalytic domain contains:

(a) an amino acid other than lysine or arginine at the position corresponding to position 698 of SEQ ID NO:1;
(b) an amino acid other than lysine or arginine at the position corresponding to position 708 of SEQ ID NO:1;
(c) an amino acid other than lysine, arginine, alanine, or glutamate at the position corresponding to position 719 of SEQ ID NO:1;
(d) an amino acid other than lysine or arginine at the position corresponding to position 698 of SEQ ID NO:1 and an amino acid other than lysine or arginine at the position corresponding to position 708 of SEQ ID NO:1;
(e) an amino acid other than lysine or arginine at the position corresponding to position 698 of SEQ ID NO:1, an amino acid other than lysine or arginine at the position corresponding to position 708 of SEQ ID NO:1, and an amino acid other than lysine or arginine at the position corresponding to position 719 of SEQ ID NO:1;
(f) an amino acid other than lysine or arginine at the position corresponding to position 698 of SEQ ID NO:1 and an amino acid other than lysine or arginine at the position corresponding to position 719 of SEQ ID NO:1; or
(g) an amino acid other than lysine or arginine at the position corresponding to position 708 of SEQ ID NO:1 and an amino acid other than lysine or arginine at the position corresponding to position 719 of SEQ ID NO:1.

32. The proteolytically active or reversibly inactivated plasmin variant of claim **30**, wherein the mammalian plasmin light chain is a human plasmin light chain.

33. The activatable plasminogen variant of claim **31**, wherein the mammalian plasminogen is a human plasminogen.

34. A proteolytically active or reversibly inactivated plasmin variant comprising a heavy chain and a light chain, wherein the heavy chain comprises amino acids 543-561 of SEQ ID NO:1 and the light chain comprises a human plasmin light chain amino acid sequence, with:

(a) an amino acid other than lysine at the position corresponding to position 698 of SEQ ID NO:1;
(b) an amino acid other than lysine at the position corresponding to position 708 of SEQ ID NO:1; or
(c) an amino acid other than arginine, alanine, or glutamate at the position corresponding to position 719 of SEQ ID NO:1,
(d) an amino acid other than lysine or arginine at the position corresponding to position 698 of SEQ ID NO:1 and an amino acid other than lysine or arginine at the position corresponding to position 708 of SEQ ID NO:1;
(e) an amino acid other than lysine or arginine at the position corresponding to position 698 of SEQ ID NO:1, an amino acid other than lysine or arginine at the position corresponding to position 708 of SEQ ID NO:1, and an amino acid other than lysine or arginine at the position corresponding to position 719 of SEQ ID NO:1;
(f) an amino acid other than lysine or arginine at the position corresponding to position 698 of SEQ ID NO:1 and an amino acid other than lysine or arginine at the position corresponding to position 719 of SEQ ID NO:1; or
(g) an amino acid other than lysine or arginine at the position corresponding to position 708 of SEQ ID NO:1 and an amino acid other than lysine or arginine at the position corresponding to position 719 of SEQ ID NO:1.

35. A method of inducing liquefaction of the vitreous in an eye in a human subject in need thereof, the method comprising administering to the eye of the human subject an effective amount of the pharmaceutical composition of claim **22**.

36. A method of inducing liquefaction of the vitreous in an eye in a human subject in need thereof, the method comprising administering to the eye of the human subject an effective amount of the pharmaceutical composition of claim **23**.

37. The proteolytically active or reversibly inactivated plasmin variant of claim **30**, wherein the amino acid at the position corresponding to position 698 of SEQ ID NO:1 of the mammalian plasmin light chain is Ala, Glu, Phe, His, Ile, Met, or Gln.

38. The proteolytically active or reversibly inactivated plasmin variant of claim **30**, wherein the amino acid at the position corresponding to position 708 of SEQ ID NO:1 of the mammalian plasmin light chain is Ala, Glu, Gln, His, Ile or Phe.

39. The proteolytically active or reversibly inactivated plasmin variant of claim **30**, wherein the amino acid at the position corresponding to position 719 of SEQ ID NO:1 of the mammalian plasmin light chain is Gln, Ile, Phe or His.

40. The proteolytically active or reversibly inactivated plasmin variant of claim **30**, wherein the plasmin variant is a Glu-plasmin variant, a Lys-plasmin variant, a midiplasmin variant, a miniplasmin variant, a microplasmin variant, or a delta-plasmin variant.

41. A pharmaceutical composition comprising the proteolytically active or reversibly inactivated plasmin variant of claim **30**, and a pharmaceutically acceptable diluent, carrier, or adjuvant.

42. The activatable plasminogen variant of claim **31**, wherein the amino acid at the position corresponding to position 698 of SEQ ID NO:1 of the light chain is Ala, Glu, Phe, His, Ile, Met, or Gln.

43. The activatable plasminogen variant of claim **31**, wherein the amino acid at the position corresponding to position 708 of SEQ ID NO:1 of the light chain is Ala, Glu, Gln, His, Ile or Phe.

44. The activatable plasminogen variant of claim **31**, wherein the amino acid at the position corresponding to position 719 of SEQ ID NO:1 of the light chain is Gln, Ile, Phe or His.

45. The activatable plasminogen variant of claim **31**, wherein the plasminogen variant is a Glu- plasminogen variant, a Lys- plasminogen variant, a midiplasminogen variant, a miniplasminogen variant, a microplasminogen variant, or a delta-plasminogen variant.

46. A pharmaceutical composition comprising the activatable plasminogen variant of claim **31**, and a pharmaceutically acceptable diluent, carrier, or adjuvant.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 9,226,953 B2
APPLICATION NO. : 13/383086
DATED : January 5, 2016
INVENTOR(S) : Richard Reinier Zwaal

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title Page, item (56) (Other Publications), Line 8: Delete "Biochemisty," and insert -- Biochemistry, --.

Title Page, item (57) (Abstract), Line 3: Delete "autocatylic" and insert -- autocatalytic --.

Specification

Column 6, Line 25: Delete "at position which" and insert -- at position 1 which --.

Column 8, Line 11: Delete "mulation two" and insert -- mulation of two --.

Column 10, Line 4: Delete "H is" and insert -- His --.

Column 12, Line 15: Delete "H is" and insert -- His --.

Column 12, Line 27: Delete "H is" and insert -- His --.

Column 15, Line 54: Delete "of" and insert -- or --.

Column 20, Line 51: Delete "TGF-13" and insert -- TGF-β --.

Column 25, Line 58 (Table 2, pre-peak 1): Delete "APDFDX(C)GKPQ" and insert -- APSFDX(C)GKPQ --.

Column 28, Line 16: Delete "H is" and insert -- His --.

Column 28, Line 24: Delete "H is" and insert -- His --.

Column 28, Line 39: Delete "Menko" and insert -- Menlo --.

Column 30, Line 50: Delete "<u>CTG***CAC***</u>" and insert -- <u>CTG***CAG***</u> --.

Column 35, Line 9: Delete ", as well as the efficacy".

Column 36, Line 1: Delete ", as well as the efficacy".

Column 36, Line 8: Delete ", as well as the efficacy".

Claims

Column 129, Line 3 (Claim 13): Delete "R15811719" and insert -- 719 --.

Signed and Sealed this
Nineteenth Day of April, 2016

Michelle K. Lee

Michelle K. Lee
Director of the United States Patent and Trademark Office